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(71) Applicant: BIOPROBE INTERNATIONAL, INC. [US/US]; 14272 Franklin Avenue, Tustin, CA 92689-7027 (US).

(72) Inventors: NGO, That, T. ; 20 Sandstone, Irvine, CA 92714 (US). BRILLHART, Knrt, L. ; 21594 Cabrosa, Mission Viejo, CA 92691 (US).

(74) Agent: BERLINER, Robert Nilsson, Robbins, Dalgarn, Berliner, Carson & Wurst; 201 North Figueroa Street, 5th Floor, Los Angeles, CA 90012 (US).

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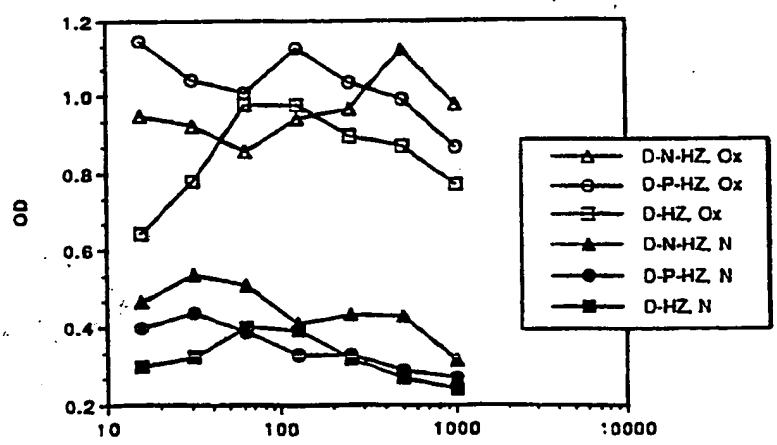

(57) Abstract

Water-s luble compounds (both monomers and polymers) including hydrophobic moieties that bind tightly to solid phase materials. These compounds carry reactive functi nal groups (e.g., hydrazide or 2-(N-methylpyridil) groups) which form stable covalent bonds with ligands at specific sites on the ligands (for example, the oligosaccharide components of immunoglobulins) that do not affect interaction of the ligand with the target m lecule. In this manner, a number of functional binding sites available for binding f suitable ligands for use in assay methods inv lving solid phase materials are provided, while reducing undesired nonspecific binding f target m lecules to the solid phase.

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COMPOSITIONS AND METHODS FOR ENHANCED BINDING IN BIOLOGICAL ASSAYS

Background of the Invention

This invention relates to methods and compositions for use in performing qualitative and quantitative chemical assays, for example of biologically active molecules. In particular, the present invention relates to methods and compositions for preparation of assay systems, particularly those including a solid phase.

Immobilization of biomolecules has played a pivotal role in the development of bioanalytical systems. Traditional enzyme immunoassay (EIA) relies on the immobilization of either antigen or antibody to a solid phase (typically, a polystyrene 96 well microtiter plate or a polystyrene bead). This immobilization is accomplished by utilizing the tendency of such plastics to bind proteins nonspecifically. While not fully understood, it is believed that such nonspecific binding involves primarily hydrophobic interactions. These tend to denature protein ligands. Further, the lack of site specificity inherent in this mechanism necessarily leads to the potential blocking of some sites of interaction with ligands of actual or potential interest.

The demand for increas d sensitivity in assays has led to the development of several different plastic formulations that have an increased capacity to bind various proteins, and in particular immunoglobulins. For example, polystyrene can be aminated by treatment with nitric acid, followed by reduction. Once chemically activated, these groups can form covalent bonds with immunoglobulins. The lack of antigen binding enhancement found with such activated polystyrenes suggests that a significant denaturation of the antibody still occurs. As an alternative, a coating for polystyrene consisting of a random copolymer of lysine and phenylalanine has been proposed. After coating, the lysine side chains were treated with excess pentane-1,5-dial to produce reactive aldehyde groups. A large increase in antigen binding activity was found using polystyrene matrices coated in this manner. Nonetheless, the expense of the polymer, the time required to produce an effective coating, and the lack of site specificity in the coupling chemistry limit its utility [Wood, W.G. and Gadow, A., J. Clin. Chem. Clin. Biochem. 21, 789-797 (1983)].

Other strategies for increasing the amount of functional IgG on the solid phase have included the use of covalently immobilized IgG binding proteins for binding the IgG of interest [Kakabakos, S.E. et al., J. Immunol. Methods 127, 147-148 (1990)]. Pretreatment of the coating IgG with low pH, followed by nonspecific binding to scrupulously cleaned polystyrene [Kakabakos, S.E. et al., Clin. Chem. 36/3, 497-500 (1990)] or acrylonitrile-butadienestyrene [Kakabakos, S.E. et al., Clin. Chem. 36/3, 492-496 (1990)] has also been explored. Additionally, several investigators have utilized activated hydrophobic

moieties [Ampon and M ans* (1988)] or fluorocarbons [Eveleigh*; Kobos and Eveleigh*] to covalently modify proteins and enhance their binding to hydrophobic surfaces and Teflon, respectively. None of these approaches, however, has been entirely successful.

It has heretofore been reported that oxidized immunoglobulins can form stable covalent bonds, at specific sites that do not affect antigen/antibody interaction, with hydrazide groups [O'Shannessy, D.J. and Hoffman, W.L., Biotech. Applied Biochem. 9, 488-496 (1987); O'Shannessy, D.J. and Quarles, R.H., J. Immunol. Methods 99, 153-161 (1987); Hoffman, W.L. and O'Shannessy, D.J., J. Immunol. Methods 112, 113-120 (1988); Cress, M.C. and Ngo, T.T., Amer. Biotech. Lab. 7(2), 16-19 (1989)].

In spite of the advances achieved to date, there remains a need for techniques which permit the purification of compositions of chemical and biological interest by exploiting the advantages inherent in using a solid phase while minimizing the degree of nonspecific binding.

It is therefore an object of the present invention to provide methods and compositions which may be employed in conjunction with solid phase materials with a wide range of ligands for use in qualitative and quantitative assays.

Summary of the Invention

In accordance with the present invention, to generate a number of functional binding sites available for binding of suitable ligands for use in assay methods involving solid phase materials while reducing undesired nonspecific binding of target molecules to the solid phase, water-soluble compounds (both monomers and polymers) including hydrophobic moieties that bind tightly to, e.g., the plastics

commonly used as solid phas s are exploited. These compounds further carry reactive functional groups (e.g., hydrazide or 2-(N-methylpyridyl) groups) which form stable covalent bonds with ligands at specific sites on the ligands (for example, the oligosaccharide components of immunoglobulins) that do not affect interaction of the ligand with the target molecule.

In particular, the present inventions provides for a class of novel reagents of the general formula $(R^H-)_x\ A\ (-X)_y$,

wherein R^H represents a hydrophobic moiety which develops substantial nonspecific interactions with a hydrophobic material, such as solid phase materials heretofore employed in assay methods;

A is an organic spacer group;

X represents a reactive group to be used in coupling a ligand for use in the particular assay method contemplated; and

each of x and y is an integer from 1 to about 1300.

In one preferred embodiment, the organic spacer group is a polymer which may accommodate a plurality of hydrophobic R^H groups and reactive groups (i.e., x and/or y >1). In a further preferred embodiment, the reactive group is a hydrazide or 2-(N-methylpyridyl) group for use in coupling an antibody or antigen as heretofore employed in conventional immunoassay procedures.

Brief Description of the Drawings

Fig. 1 illustrates the binding of horseradish peroxidase (HRP) to untreated (N) and oxidized (Ox) IgG specific for HRP applied to microtiter plates variously coated with inventive and control compositions.

Figs. 2A - 2G compare the levels of binding of HRP to normal and oxidized IgG specific for HRP on medium and high binding microtiter plates which are either uncoated or coated with a composition in accordance with the instant invention.

Fig. 3 compares the levels of binding of HRP with coated polystyrene beads using no IgG, unoxidized IgG and oxidized IgG specific for HRP.

Figs. 4A and 4B compare the levels of binding of HRP with uncoated and coated polyethylene (Fig. 4A) and polystyrene (Fig. 4B) beads and with unoxidized and oxidized IgG.

Fig. 5 compares the levels of HRP binding to oxidized and unoxidized IgG bound to polystyrene microtiter plates which are untreated or coated with various derivatives of polyacrylamide and polyacrylic acid.

Fig. 6 illustrates the levels of BSA binding to microtiter plates treated with activated polyvinylalcohol treated with various concentrations of phenylhydrazine.

Fig. 7 illustrates the levels of binding of ß-galactosidase to polystyrene microtiter plates which are provided with PVA-MP and PVA-PBA-MP coatings.

Fig. 8 illustrates the levels of binding of ß-galactosidase to untreated tissue culture plates and plates treated with polyvinyl alcohol-methylpyridone and polyvinyl alcohol-phenylbutylamine-methylpyridone conjugates.

Fig. 9 illustrates the overall synthetic route for preparation of dextran-phenyl-dithiopyridine and dextran-phenyl maleimide conjugates.

Fig. 10 illustrates the levels of ß-galactosidase activity observed on polystyrene microtiter plates which are either untreated or coated

with dextran-phenyl-dithiopyridine or dextran-phenyl maleimide conjugates.

Fig. 11 illustrates the levels of binding of rabbit anti-HRP on polystyrene microtiter plates which are untreated or coated with dextran-aldehyde and dextran-phenyl-aldehyde conjugates.

Fig. 12 illustrates the levels of HRP binding to rabbit antiperoxidase on polystyrene microtiter plates which are untreated or coated with dextran-aldehyde and dextran-phenyl aldehyde conjugates.

Fig. 13 illustrates the levels of HRP binding to rabbit anti-peroxidase on polystyrene microtiter plates which are untreated or coated with dextran-phenyl-NHS ester.

Fig. 14 illustrates the overall synthetic route for preparation of fetuin-hydrazide and dinitrophenyl-fetuin-hydrazide conjugates.

Fig. 15 illustrates the levels of binding of peroxidase to untreated microtiter plates and to oxidized or untreated rabbit antiperoxidase on plates coated with fetuin-hydrazide and dinitrophenyl-fetuin-hydrazide conjugates.

Fig. 16 illustrates the overall synthetic route for preparation of monomeric hydrophobe-hydrazide conjugates.

Fig. 17 illustrates the levels of peroxidase binding activity to oxidized rabbit antiperoxidase on microtiter plates which are untreated or coated with monomeric hydrophobe-hydrazide conjugates.

Detailed Description of the Invention

Pursuant to the present invention, many of the problems encountered employing, for example, solid phase materials such as polystyrene microtiter plates or beads in assay methods are substantially obviated.

The desired coating material is provided in a hybrid form as a combination of (1) one or more hydrophobic moieties designed to maximize and therefore stabilize the nonspecific binding of the coating material with the solid phase materials, and (2) reactive groups capable of forming stable covalent bonds with ligand of interest in a specific manner. Thus, the ligands are firmly attached to the solid phase via the coating materials. Furthermore, any effects of nonspecific (e.g., hydrophobic) interactions on the properties of the coating materials with the solid phase minimize or eliminated the nonspecific binding of ligands to the solid phase. The nonspecific binding sites of the solid phase material are preferentially occupied by the hydrophobic moieties of the coating material, while the reactive group is substantially free from interfering interactions with the hydrophobic materials and thus can react more readily with a particular ligand.

In order to optimize the orientation of the ligand away from the hydrophobic material, a suitable spacer group is generally interposed between the reactive group and the hydrophobic moiety. This spacer serves the purpose of effectively establishing two domains in the molecule: a hydrophobic domain which interacts with the hydrophobic materials (primarily, through nonspecific binding mechanisms); and a reactive group-presenting domain, which permits the binding to specific site(s) of the ligand in readily-accessible form. In those instances where the ligand itself is substantially water-soluble and provides one or more functional groups for attachment of the hydrophobic moiety at some distance from the binding site(s), the spacer/reactive group combination may comprise a simple bifunctional molecule which

permits attachment of the hydrophobic moiety directly to the reactive group. For example, a dihydrazide (such as adipic dihydrazide) may be used to connect a hydrophobic aldehyde with a water-soluble ligand with available aldehyde groups (e.g., a polysaccharide wherein at least one vicinal diol group has been oxidized to the dialdehyde form). In other cases, the spacer may additionally serve the purpose of imparting to the molecule as a whole the property of water solubility. Because the attachment to the solid phase and the presentation of the reactive group to the target molecule effectively requires that the molecule comprise both a hydrophobic domain (for binding to what may be considered the hydrophobic solid phase) and a hydrophilic domain (for binding the ligand via the reactive group and subsequently, the target molecule to the ligand during the course of the assay typically carried out in aqueous solution), it is important that the reactive group itself or the combination of reactive group with the spacer molecule exhibits a sufficient difference in hydrophobic character such that an orientation of the molecule to present the reactive group (or any ligand subsequently attached thereto) may be effected.

Depending on the nature of the spacer and the ligand(s), a wide variety of reactive groups may suitably be employed in accordance with the present invention. Representative reactive groups include the following: hydrazide, N-methylpyridyl, tresyl, aldehyde, haloacetyl, active esters of carboxylic acids, maleimido, imidate, epoxide, thiol, and dicarbonyl [Means, G.E. and Feeney, R.E., Bioconjugate Chem. 1, 2-12 (1990)]. Other reactive groups which may be employed in conjunction with a specific ligand or type of ligand as would be apparent to those of

skill in the art are also contemplated as within the scope of the present invention.

In accordance with one preferred embodiment of the invention, the spacer group comprises a substantially water-soluble polymeric backbone to which a plurality of hydrophobic moieties and a plurality of reactive groups may be attached. Suitably, the polymeric backbone contains a plurality of aldehyde functions (or functional groups which may be converted to aldehydes). These aldehydes may be obtained by oxidation of carbohydrates; alternatively, chemical grafting methods may be employed to provide suitable starting materials. Both 2-fluoro-1-methylpyridinium salt activated hydroxyl groups and active esters of carboxylic acid have also been used. Hydrophobic moieties (e.g., aromatic residues such as phenyl or naphthyl), for example using the corresponding hydrazides, may then be reacted with some of the free aldehyde functions to form the corresponding hydrazones. In an analogous manner, attachment of suitable ligand to the polymeric material by reaction with other free aldehydes (for example, via a reactive group-hydrazine intermediate or by reaction of the polymeric material with a dihydrazide followed by addition of suitable ligand) may also be effected.

In accordance with one preferred embodiment of the present invention, the ligand comprises a biologically active molecule containing one or more carbohydrate residues which may be oxidized to provide aldehyde groups for reaction with free hydrazide groups. Such biologically active materials include glycoproteins, polysaccharides, glycolipids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Particularly preferred for purpos s of the

pres nt invention are immunoglobulins, which have been extensively exploited in a wide variety of immunoassay methods. The immunoglobulins are oriented away from the solid medium through nonspecific binding of a hydrophobic residue connected to the immunoglobulin via its carbohydrate residue.

In accordance with a preferred embodiment of the invention, aldehyde-dextran is employed as a suitable polymeric material to which both the hydrophobic residues and reactive groups are attached through conventional hydrazide chemistry. Other polymers, such as hydrolyzed polyacrylamide, polyacrylic acid, polyvinyl alcohol, and polypeptides, may also be used. Whereas the direct reaction of a reactive dihydrazide (e.g., adipic dihydrazide) tends to result in the formation of insoluble products (probably due to crosslinking), the addition of small amounts of hydrophobic hydrazides to the dihydrazide reaction mixture tends to inhibit crosslinking, permitting a longer reaction time and a more complete reaction. Measurement of hydrazide concentration indicate that there is an average of one hydrazide group for every 2.5 glucose subunits in the exemplary dextran polymers, which is an extremely high degree of substitution.

Pursuant to this preferred embodiment of the invention, there is a large increase in antigen binding capacity when compared to uncoated matrices (i.e., matrices wherein the immunoglobulin is bound directly by nonspecific interactions), using oxidized IgG. The IgG is immobilized in the preferred orientation by reaction with the free hydrazides on the polymeric material. As the polymers are designed to bind to th solid phase by hydrophobic

interactions, hydrazide groups may b placed on a variety of plastics quickly and easily by adsorption.

Particular advantages of this embodiment of the invention include the following: (1) an increase in binding capacity of the coated solid phase; (2) the coating procedure is simple, mild and not damaging to the surface of the solid phase; and (3) nonspecific binding of unmodified IgG is greatly reduced when high binding solid phase formulations (e.g., high binding polystyrene plates or beads) are coated with the novel polymeric materials. Thus, the inventive compositions can be used simultaneously to increase the amount of antigen captured and to block the high degree of nonspecific binding of antigen which currently limits the use of high binding microtiter plates in immunoassay methods.

The use of polymers to introduce hydrazine groups onto solid phase materials allows the site-specific immobilization of a wide range of other oxidized glycoproteins and polysaccharide antigens. Antigens that do not contain aldehyde groups could be immobilized using surfaces activated by coating with hydrazide-containing polymers followed by reaction with suitable crosslinkers (e.g., dialdehydes). Alternatively, antigens could be biotinylated and then immobilized by pretreating the solid phase with a dextran-adipic acid hydrazide coating and oxidized avidin (a glycoprotein). Polymers that bind tightly to plastics and form covalent bonds with the nucleophilic groups of proteins (e.g., -amino or -SH groups) are also contemplated.

The invention will be better understood by reference to the following examples which are intended for purposes of illustration and are not to be construed as in any way limiting the scope of the

present invention, which is d fined in the claims appended hereto.

EXAMPLES

As employed in the following examples, dextran (MW ≈ 20 kD), rabbit anti-horseradish peroxidase, and horseradish peroxidase were purchased from the Sigma Chemical Company. Sodium meta-periodate, adipic acid dihydrazide, phenylhydrazine, and sodium borohydride were purchased from the Fluka Chemical Company. 1-Naphthalene-acethydrazide was purchased from Lancaster Synthesis Ltd. An AvidChrom cartridge containing immobilized natural Protein A and 5 ml desalting cartridges were from BioProbe International, Inc. Microtiter plates were obtained from Costar (polystyrene), Dynatech (polystyrene), Falcon (polyvinylchloride), and Nunc (polystyrene). Polystyrene beads were obtained from the Precision Plastic Ball Company (0.25 inch) and Hybritech (0.31 inch); linear polyethylene beads (0.25 inch) were obtained from the Precision Plastic Ball Company.

Example 1

Preparation of the Soluble Polymeric Hydrazide (SPH)

Aldehyde-dextran was prepared by gradually adding dry sodium meta-periodate to a 10% solution of dextran (in 50 mM acetate buffer, pH 5.0) with rapid stirring over 15 minutes to give a final concentration of 0.56 M periodate. An ice bath was used to maintain the temperature of the reaction mixture at 25°C for the first 30 minutes of the reaction, after which the mixture was placed in the dark. After 6 hours the reaction was terminated by adding excess ethylene glycol and dialyzing against 50 mM acetate buffer (pH 5.0).

Dextran-adipic hydrazide (D-HZ) was produced by diluting this aldehyde-dextran to 5 mg ml^{-1} in

acetate buff r and adding it slowly to an equivalen volume of 50 mg ml^{-1} adipic acid dihydrazide in acetate buffer with rapid stirring. After 6 hours at 25°C, the reaction was terminated by raising the pH to 9.5 with 10 N NaOH.

Dextran-adipic hydrazide containing hydrophobic groups was prepared in a similar manner; the aldehyde dextran was added slowly to adipic acid dihydrazide:phenylhydrazine (500:1) or adipic acid dihydrazide:napthaleneacethydrazide (25:1) in acetate buffer. Napthaleneacethydrazide was dissolved in a small volume of dimethyl sulfoxide prior to use. This reaction was terminated after 16 hours at 25°C by raising the pH to 9.5. Unless otherwise noted the polymer was treated with excess sodium borohydride for 16 hours at 25°C to reduce Schiff bases and residual aldehyde groups. After reducing the pH to 3 for 1 hour to eliminate residual $NaBH_4$, the polymers were dialyzed extensively against 50 mM carbonate buffer (pH 9.5) to recover the respective dextran-phenyl-adipic hydrazide (D-P-HZ) and dextran-naphthyl-adipic hydrazide (D-N-HZ) products.

Hydrazide content was estimated by mixing 25 μl of a 1 mg ml^{-1} solution of the polymer with 1 ml of trinitrobenzene-sulfonic acid (10 mg ml^{-1} in 50 mM carbonate, pH 9.5), incubating for 1 hour at 25°C in the dark, and measuring the absorbance at 500 nm (E_{500nm} = 15,600 AU $M^{-1}cm^{-1}$).

Example 2

Enhancement of IgG Binding to Coated Microtiter Plates

The dextran-adipic hydrazide polymers prepared in accordance with the method of Example 1 were applied to various matrices in 50 mM carbonate buff r (100 μl per well in this example or 1 ml per bead in Example 3). After 30 minutes to 16 hours at

37°C the solid phase was washed 3 tim s, 5 minutes per wash, with deionized water (200 µl per well in this example or 3 ml per bead in Example 3).

IgG was isolated from fractionated rabbit antiserum specific for horseradish peroxidase using Protein A-Fractogel according to the manufacturer's directions. This was dialyzed against 50 mM acetate, pH 5.0, then oxidized by adding a 1/10 volume of freshly prepared 100 mM sodium meta-periodate in acetate buffer and incubating for 30 minutes at 25°C in the dark. The reaction was terminated by adding excess ethylene glycol (10 µl ml^{-1}) and either dialyzing the reaction mixture against acetate buffer or using a 5 ml desalting cartridge. Human IgG was oxidized in a similar manner.

Either untreated or oxidized IgG was then applied in acetate buffer. After 16 hours at 5°C the solid phase was washed with 100 mM Tris + 0.1% Tween-20 (pH 7.5, wash buffer), then blocked by adding 10 mg ml^{-1} BSA in wash buffer (blocking buffer). After 15 minutes the blocking buffer was removed and HRP applied in blocking buffer (100 µl per well or 1 ml per bead); after 1 hour at 25°C the matrix was again washed with washing buffer and a commercial tetramethylbenzidine (TMB) substrate mixture added. After 5 minutes at 25°C the reaction was stopped either by pipetting the substrate from the matrix or by adding 2.5 M H_2SO_4 to a final concentration of 0.5 M. The absorbance was then measured at 650 nm or 450 nm, respectively.

As shown in Fig. 1, coating polystyrene microtiter plates with dextran-adipic hydrazide polymers at all concentrations strongly enhances the binding of HRP by immobilized oxidized antibody to HRP. The assays were performed using Nunc Maxisorb

(polystyren) microtiter plat s and rabbit anti-HRP diluted 1:9 with human IgG at a final concentration of 50 μg IgG in a volume of 100 μm per well. HRP was applied at 1 μg. Oxidized IgG is indicated by open points, untreated IgG by filled points. The dextran coatings in this experiment were not reduced with sodium borohydride. All values are the means of triplicate wells.

As is apparent from Fig. 1, there is a defined concentration of soluble polymeric hydrazide which gives optimal binding activity of the immobilized antibody. At high coating concentrations the binding of oxidized IgG is reduced, probably due to layering of the polymer coating. The simplest polymer coating, dextran-adipic hydrazide, shows a sharp drop in oxidized IgG binding at low (< 50 μg per ml) concentrations. This effect is not seen with dextran-adipic hydrazide polymers that contain hydrophobic groups. The optimal coating concentration for dextran-adipic hydrazide was chosen as 200 $\mu g\ ml^{-1}$; the optimal concentration for dextran-adipic hydrazide with hydrophobic groups was chosen as 100 $\mu g\ ml^{-1}$.

Figs. 2A - 2F illustrate the results of tests to determine the degree of enhancement of IgG binding achieved pursuant to the present invention. Figs. 2A and 2B illustrate the results using Nunc General Purpose (Medium binding) and Maxisorb™ (High Binding) Polystyrene Microtiter plates, respectively. Figs. 2C and 2D illustrate the results using Dynatech Immulon 1™ (Medium binding) and Immulon 2™ (High binding) Polystyrene Microtiter plates, respectively. Figs. 2E and 2F illustrate the results using Costar Medium Binding and High Binding Polystyrene Microtiter plates, respectiv ly. Fig. 2G illustrates the results using Falcon Microtest III™

Polyvinylchl rid Micr titer plates.

In all of the comparisons reported in Figs. 2A-2G, dextran-phenyl-adipic hydrazide (100 μg per ml in 50 mM carbonate, pH 9.5) was applied at 100 μl per well and incubated for 30 minutes at 37°C. After thorough washing with deionized water, 100 μl of either oxidized or unoxidized rabbit IgG specific for horseradish peroxidase (diluted 1:9 with normal IgG) was applied in 50 nM acetate (pH 5.0) at the indicated concentrations and incubated for 16 hours at 5°C. After washing and blocking (as previously described), a large excess of HRP (100 μl of 10 μg per ml) was applied in blocking buffer. After washing (as previously described), bound HRP activity was measured using a commercial TMB substrate mixture and H_2SO_4. The values reported represent the means of triplicate experiments.

As shown in Figs. 2A though 2F, both medium binding (Figs. 2A, 2C, and 2E) and high binding (Figs. 2B, 2D, and 2F) polystyrene microtiter plates coated with the dextran-phenyl-adipic hydrazide polymer and oxidized rabbit anti-HRP show greatly enhanced binding of the antigen compared to uncoated plates and coated plates treated with unoxidized IgG. Coating of high binding polystyrene formulations reduced the binding of unmodified IgG; this effect was not significant with medium binding formulations. Flexible polyvinylchloride microtiter plates (Fig. 2G) show an enhancement of antigen binding capacity similar to that observed for high binding polystyrene formulations.

Example 3

Enhancement of IgG Binding to Coated Beads

Dextran-phenyl-adipic hydrazide (100 μg per ml in 50 mM carbonate, pH 9.5) prepared in accordance

with the method of Example 1 was applied to polystyrene and polyethylene beads at 1 ml per bead and incubated for 30 minutes at 37°C. After thorough washing with deionized water 1 ml per bead of either oxidized or unoxidized rabbit IgG specific for horseradish peroxidase (diluted 1:9 with normal IgG) was applied in 50 mM acetate (pH 5.0) at the concentrations indicated in Figs. 3, 4A and 4B and incubated for 16 hours at 5°C. After washing and blocking (as previously described), bound HRP activity was measured using a commercial TMB substrate mixture. The reaction was stopped by removal of the bead. The values reported represent the means of triplicate experiments.

Fig. 3 reports the results obtained using coated Precision Ball Polystyrene beads and HRP only, and HRP with unoxidized IgG or oxidized IgG. Figs. 4A and 4B report the results using both oxidized and unoxidized IgG with coated and uncoated Hybritech Polystyrene (Fig. 4A) and Precision Ball Polyethylene (Fig. 4B) beads, respectively.

Polystyrene beads coated with the dextran-phenyl-adipic hydrazide polymer and oxidized antibody show a greatly increased ability to bind antigen (Figs. 3 and 4A). Coated beads reacted with oxidized anti-HRP typically bound ≈4 times more HRP than uncoated beads or coated beads treated with unoxidized IgG. Similarly, coated linear polyethylene beads reacted with oxidized IgG bound approximately twice as much antigen as either uncoated beads or coated beads treated with unoxidized IgG (Fig. 4B).

Example 4

Enhancement of IgG Binding to Polystyrene Microtiter Plates Coated with Modified Polyacrylamide and Polyacrylic Acid

Nunc General Purpose (Medium Binding) Microtiter plates were coated with 100 µl of 100 µg per ml of adipic hydrazide labeled polyacrylamide or polyacrylic acid in 50 mM sodium carbonate (pH 9.5) for 30 minutes at 37°C. After washing with ionized water three times for a duration of 5 minutes per wash, the plates were treated with rabbit anti-horseradish peroxidase and assayed for peroxidase binding as described in Example 2. Little change in antigen binding activity is seen on the addition of hydrophobic groups to the polymer; presumably, this is due to the very hydrophobic nature of the polymeric backbones.

The results of these assays are shown in the form of a pictogram (Fig. 5) and are reported in Table 1. The entries in Fig. 5 and Table 1 represent the following:

A: Polymeric coating material prepared by reacting 14 mM polyacrylic acid (PAA; MW = 100 kD) with 140 mM adipic acid dihydrazide (ADH) in the presence of ethyldimethylaminopropylcarbodiimide (EDC) at pH 4.5-5.5 for 16 hours at room temperature;

B: Polymeric coating material prepared as in A with addition of 0.3 mM phenylhydrazine to the mixture [PAA + ADH:phenylhydrazine (50:1) + EDC];

C: Polymeric coating material prepared as in B, but with 3.0 mM phenylhydrazine;

D: Polymeric coating material prepared as in A, using hydrolyzed polyacrylamide (PAM; 200 kD) [PAM + ADH + EDC];

E: Polymeric coating material prepared as in C, using hydrolyzed polyacrylamide

(200 kD) [PAM + ADH:phenylhydrazine (50:1) + ADC]; and

F: Untreated plate.

In Table 1, peroxidase binding is expressed as a percentage of the maximum observed binding.

TABLE 1

Polymer	Unoxidized IgG	Oxidized IgG
A	13.96%	100.00%
B	10.70%	93.76%
C	13.93%	96.88%
D	12.97%	85.54%
E	13.11%	78.31%
none	9.74%	14.08%

Example 5
Enhancement of Dextran-Hydrazide Binding Stability by Addition of Hydrophobic Groups

Dextran-Hydrazide (D-HZ) and Dextran-Phenylhydrazide (D-P-HZ; D-P-HZ:ADH = 1:500) prepared according to Example 1 were coated onto a polystyrene microtiter plate as in Example 2. The samples were then treated for 1 hour at room temperature with varying concentrations of Tween-20. After washing six times with deionized water, the plates were treated with oxidized rabbit anti-HRP and HRP as previously described to evaluated antigen binding capacity. The polymer carrying hydrophobic groups (i.e., D-P-HZ) showed greater resistance to detergent treatment, indicating that the addition of hydrophobic groups stabilizes binding of the polymer to polystyrene. HRP binding is reported in Table 2 as a percentage of the maximum observed binding activity.

TABLE 2

[Tween-20],%	D-HZ coated plates	D-Phe-HZ coated plates
10.00	26.91%	66.74%
5.00	29.98%	86.21%
2.50	38.07%	82.28%
1.25	42.67%	93.00%
0.63	48.25%	98.03%
0.31	82.39%	100.00%

In a further sequence of comparisons, dextran-phenylhydrazides prepared as in Example 1 in the presence of varying concentrations of phenylhydrazine were coated onto polystyrene microtiter plates as in Example 2. Half of the wells were then treated with 100 µl of 1% Tween-20 for one hour at room temperature. After washing six times with deionized water, the plates were treated with oxidized rabbit anti-HRP and HRP as previously described to evaluate antigen binding capacity. Optimal resistance to detergent treatment (and, presumably, maximum stability of the polymer:polystyrene interaction) is seen at a phenylhydrazine concentration in the starting materials of 30 µM; nonetheless, as reported in Table 3, far lower concentrations of phenylhydrazine also markedly improve stability. HRP binding is reported as a percentage of the maximum observed binding activity.

TABLE 3

[Phenylhydrazide], μM	No Detergent	1% Tween-20
0	36.70%	12.66%
300	92.45%	49.26%
30	93.62%	52.23%
3	97.34%	48.94%
0.3	88.83%	46.38%
0.03	91.70%	41.60%
3×10^{-3}	94.15%	44.04%
3×10^{-4}	100.00%	44.79%

Example 6

Enhancement of Antigen Binding Capacity of Polystyrene Microtiter Plate by Coating with 2-Fluoro-a-Methylpyridinium Activated Polyvinylalcohol

Polyvinylalcohol (PVA; 100 kD) was dissolved in dimethylsulfoxide (DMSO) at 10 mg per ml and activated with 130 mM 2-fluoro-1-methylpyridinium toluene sulfonate (FMP) in the presence of 145 mM triethylamine (TEA) for two hours at room temperature. The product was precipitated with 8 volumes of acetone to give the activated PVA-MP. This was redissolved in DMSO and reacted with various concentrations of phenylhydrazine in the presence of 100 mM TEA to yield PVA-P-HZ. These polymers were dissolved in 10 mM phosphoric acid and coated onto polystyrene microtiter plates at various concentrations for 30 minutes at room temperature. The plates were then treated with 50 μg per ml rabbit anti-HRP (diluted 1:10 in normal rabbit IgG) in 50 mM bicarbonate for 1 hour at room temperature and HRP binding was evaluated as previously described. As is apparent from Fig. 6, coating of th plate with FMP-activated polyvinylalcohol markedly enhanced the binding of IgG.

PVA-MP derivatives with ph nylhydrazine showed even greater enhancement of IgG binding; the optimal phenylhydrazine concentration was determined to be 0.1 mM. The broad range of phenylhydrazine concentrations that enhance the binding of IgG probably reflect the balance between enhanced stability of the polymer binding to the plastic and the consumption of active groups.

Example 7

Binding of ß-Galactosidase to 2-Fluoro-1-Methylpyridinium-Activated Polyvinyl Alcohol

Polyvinyl alcohol (100 kD) was activated with 2-Fluoro-1-Methylpyridinium and triethylamine as in Example 6 to give polyvinylalcohol-methylpyridone (PVA-MP). Phenylbutylamine derivatives of this polymer (PVA-PBA-MP) were prepared by dissolving PVA-MP in anhydrous DMSO containing 10 mM triethylamine at 10 mg per ml and adding phenylbutylamine to give a final concentration of 1 µM. After a 16 hour incubation at 25°C most of the solvent was removed by heating the reaction mixture to 50°C in a vacùum of 25 inches of Hg for 4 hours.

PVA-MP and PVA-PBA-MP in DMSO at 10 mg per ml were mixed with 1:10 with 10 mM phosphoric acid. This was serially diluted in phosphoric acid and applied to microtiter plates at 100 µl per well. After a 30 minute incubation at 25°C, the wells were rinsed 3 times, 5 minutes each with phosphoric acid (200 µl per well). Freshly reduced ß-galactosidase (25 µg per ml in 50 mM phosphate, pH 7.5) was then applied at 100 µl per well. After a 2 hour incubation at 25°C, the wells were rinsed 3 times, 5 minutes each, with 100 mM Tris + 0.05% Tween-20, pH 7.5 (200 µl per well). 200 µl of 5mM o-nitrophenyl-ß-D-galactopyranoside was then applied to each well and

th enzyme activity monitored kinetically at 405 nm for 15 minutes. Both the PVA-MP and PVA-PBA-MP coatings markedly enhance the binding of ß-galactosidase to polystyrene microtiter plates, as illustrated in Fig. 7. This demonstrates that 2-fluoro-1-methylpyridinium-activated coating materials are suitable for enhancing the binding of thiol-containing compounds to solid phases.

Example 8

Activation of Tissue Culture Plates with Polyvinyl Alcohol-Methylpyridone and Polyvinyl Alcohol-Phenylbutylamine-Methylpyridone and Binding of ß-Galactosidase

Polyvinyl alcohol (100 kD) was activated with 2-Fluoro-1-Methylpyridinium and triethylamine as in Example 6 to give polyvinylalcohol-methylpyridone (PVA-MP). Phenylbutylamine derivatives of this polymer (PVA-PBA-MP) were prepared by dissolving PVA-MP in anhydrous DMSO containing 10 mM triethylamine at 10 mg per ml and adding phenylbutylamine to give a final concentration of 1 µM. After a 16 hour incubation at 25°C most of the solvent was removed by heating the reaction mixture to 50°C in a vacuum of 25 inches of Hg for 4 hours.

2.5 ml of the conjugates (100 µg per ml in 10 mM phosphoric acid) were placed in Corning polystyrene tissue culture plates (2.5 cm). After a 30 minute incubation at 25°C the plates were washed 3 times, 5 minutes each, with 5 ml of phosphoric acid to remove unbound conjugate. The plates were then treated with 2.5 ml of ß-galactosidase (25 µg per ml in 50 mM phosphate, pH 7.5) and incubated for 1 hour at 25°C. The plates were then washed 3 times, 5 minutes each, with phophat buffer to remove unbound ß-galactosidase and 5 ml of 5 mM o-nitrophenyl-ß-D-

galactopyranoside in phosphate buffer was added to measure enzyme activity. After 5 minutes the reaction was halted by pipetting the substrate mixture from the plates and the optical density at 405 nm was measured. As illustrated in Fig. 8, tissue culture plates treated with the conjugates bound significantly (10-20 fold) more ß-galactosidase than untreated plates, demonstrating that both polyvinyl alcohol-methylpyridone and polyvinyl alcohol-phenylbutylamine-methylpyridone can effectively activate these solid phases. They are therefore useful for the immobilization of known or suspected cell attachment factors to plastic tissue culture devices.

Example 9

Immobilization of Protein A on Porex™ Using Polyvinyl Alcohol-Phenylhydrazine-Methylpyridone and Binding of Serum IgG

Porex™ filter material was cut into 25 mm disks and placed in filter holders. Polyvinyl alcohol-phenylhydrazine-methylpyridone was prepared as in Example 6. The polymer was dissolved at 1 mg per ml in 10 mM phosphoric acid and passed through a 25 mm Porex disk at approximately 1 ml per minute for 1 hour. The Porex™ disks was then rinsed with 25 ml of phosphoric acid to remove unbound polyvinyl alcohol-phenylhydrazine-methylpyridone.

Protein A (1 mg per ml in 50 mM bicarbonate, pH 8.5) was circulated through activated and control Porex™ disks for 16 hours at room temperature at approximately 1 ml per minute. The disks were then rinsed with 25 ml of 10 mM phosphate + 150 mM NaCl (PBS, pH 7.4) followed by 25 ml of 100 mM acetate (pH 3.0) and an additional 25 ml of PBS to remove unbound protein A. Human serum was diluted 1:3 with PBS and 20 ml was applied to each Porex™ disk at approximately

1 ml p r min while monitoring the absorbance of the effluent at 280 nm. After rinsing with PBS until no further UV absorbing material was apparent, the disk was treated with 100 mM acetate, pH 3.0., to elute any bound IgG. Porex that had not been activated showed only a small, nonspecific UV peak, indicating only a small amount of IgG binding activity. The polyvinyl alcohol-phenylhydrazine-methylpyridone treated Porex™ subsequently reacted with protein A eluted a distinct UV absorbing peak upon treatment with acetate buffer. This indicates that these polymers can be used to inroduce active groups onto macroporous hydrophobic matrices, and that these matrices can be subsequently used for affinity chromatography on an analytical scale.

Example 10

Preparation and Applciation of Dextran-Phenyl-Dithiopyridine and Dextran-Phenyl Maleimide Conjugates

Dextran was oxidized with $NaIO_4$ as described in Example 1 and dialyzed against 50 mM $NaHCO_3$ pH 8.5. This dextran-aldehyde was incubated with a 10 fold molar excess of phenylhydrazine and a 1000 fold molar excess of diaminohexane for 16 Hours at 25°C. The pH of the reaction mixture was then raised to 10 using 10 N NaOH and a 100 fold molar excess of $NaBH_4$ was added to reduce Schiff bases and remaining aldehyde groups to secondary amines and hydroxyls, respectively. An additional aliquot of $NaBH_4$ was added after 1 hour and the reaction stopped after 1 hour by adjusting the pH to 3 with 6 N HCl. The resulting dextran-phenyl-amine (2 mg per ml) was dialyzed against 50 mM HEPES (pH 7) and a 1/10 volume of either 10 mM 3-(2-pyridyldithio) propionic acid N-hydroxysuccinimide ester (SPDP) in DMSO or 10 mM succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylat (SMCC) in DMSO was added and

allowed to react for 16 hours at 25°C. The overall synthetic routes are depicted in Fig. 9.

The polymers described above were serially diluted in HEPES buffer and applied to polystyrene microtiter plates at 100 µl per well. After a 30 minute incubation at 25°C the plates were washed 3 times, 5 minutes each, with deionized water and a 100 µl of ß-galactosidase (25 µg per ml in HEPES) were added to the wells. After an 1 hour incubation at 25°C, the plates were washed 3 times, 5 minutes each, with 100 mM Tris (pH 7.5) + 0.05% Tween-20 to remove unbound ß-galactosidase and the bound ß-galactosidase activity measured by adding 200 µl of 5 mM o-nitrophenyl-ß-D-galactopyranoside in PBS (10 mM phosphate + 150 mM NaCl, pH 7.5) to the wells and measuring the absorbance at 450 nm after 10 minutes. As illustrated in Fig. 10, wells coated with the thiol-reactive polymers show significantly increased binding of ß-galactosidase (as shown by increased enzyme activity) over uncoated wells in a concentration dependent manner. This demonstrates that dithiopyridine and maleimide groups are suitably and conveniently introduced to solid phases by using the polymeric conjugates. The lower enzyme activity observed at high concentrations of the coatings is probably due to layering of the polymers, resulting in stacking and/or destabilization of the coating.

Example 11

Preparation and Applciation of Dextran-Aldehyde and Dextran-Phenyl-Aldehyde Conjugates

Dextran-aldehyde was prepared by the oxidation of dextran (20 kD) with $NaIO_4$ as described in Example 1. Dextran-phenyl-aldehyde was prepared from dextran-aldehyde by reaction with a 10 fold molar excess of phenylhydrazine for 1 hour and dialyzing

against 100 mM HEPES, pH 7.5, to remove excess phenylhydrazine.

The polymeric aldehydes were serially diluted into wells of polystyrene microtiter plates (100 µl per well); after a 30 minute incubation at 25°C the plates were rinsed with deionized water (200 µl per well) for 5 minutes and 100 µl of rabbit anti-HRP (diluted 1:10 with nonspecific rabbit IgG, 50µg per ml in 10 mM phosphate + 150 mM NaCl [PBS], pH 7.4) were added to the wells. After a 1 hour incubation at 25°C 50 µl of 75 mM $NaCNBH_3$ in PBS was added to the wells to reduce Schiff bases to stable secondary amines; after 30 minutes the plates werewashed to remove unbound antiperoxidase and tested for peroxidase binding activity as described in Example 2. Stability of the coatings was measured by incubating coated wells in 100 µl of PBS for 30 minutes at 25°C followed by an additional set of deionized water washes; the results are illustrated in Fig. 11. Wells were then treated with rabbit antiperoxidase and $NaCNBH_3$ as described above and tested for peroxidase binding capactity as in Example 2. The results of these tests are depicted in Fig. 12, wherein D-aldehyde = dextran-aldehyde, D-Phealdehyde = dextran-phenyl-aldehyde. Wells treated with polymeric aldehydes bound significantly more IgG, as determined by the degree of antigen (peroxidase) binding, than untreated wells. This demosntrates that these polymers are a suitable and convenient means for the introduction of reactive aldehyde groups to solid phases. Dextran-aldehyde binds well to the polystyrene, probably due to the hydrophobic nature of the polymer; dextran-phenyl-aldehyde gives slightly less IgG binding than dextran-aldehyde, probably due to consumption of active groups. The dextran-phenyl-

aldehyde shows enhanced binding stability. The decrease in IgG binding seen at high polymer concentrations is probably due to layering and resulting destabilization of the coating material.

TABLE 4

Coating	% of Maximum Binding
None	20%
Dextran-Aldehyde	49%
Dextran-Phenyl-Aldehyde	100%

Example 12

Preparation and Application of Dextran-Phenyl-Aminocaproic Acid-N-Hydroxysuccinimide Ester

A dextran-phenyl-aminocaproic acid conjugate was prepared by oxidizing dextran with $NaIO_4$ in acetate buffer as described in Example 1 and incubating the oxidized dextran with equimolar phenylhydrazine and a 100 fold molar excess of ϵ-aminocaproic acid in 50 mM acetate buffer (pH 5) for 6 hours at 25°C. The resulting Schiff bases and remaining aldehyde groups were reduced by adjusting the pH to 10 with 10 N NaOH and incubating with a 10 fold excess of $NaBH_4$ was added. After 1 hour at 25 °C, an additional 10 fold excess of $NaBH_4$ was added. After 1 hour at 25°C, the mixture was acidified with 6 N HCl to remove residual $NaBH_4$ and then dialysed exhaustively against deionized water to remove buffer salts and unreacted compounds. The resulting dextran-phenyl-aminocaproic acid was lyophilized and redissolved in anhydrous dimethyl sulfoxide, then treated with a 2 fold molar excess of N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride. After 2 hours at 25°C most of the solvent was evaporate in vacuo at 50°C to give a 20 mg per ml solution.

Dextran-phenyl-aminocaproic acid-N-Hydroxysuccinimide ester conjugate in DMSO was serially diluted in 10mM phosphoric acid for application to microtiter plates (100 µl per well). After a 30 minute incubation at 25°C the plates were washed with deionized water (200 µl per well) for 5 minutes and rabbit anti-peroxidase (diluted 1:10 with nonspecific rabbit IgG, 50 µg per ml) applied in 100 mM HEPES (pH 7.5, 100 µl per well). After a 2 hour incubation at 25°C, the plates were washed to removed unbound antiperoxidase and peroxidase binding was measured as described in Example 2. Wells coated with the dextran-phenyl-NHS ester showed significantly higher binding of IgG, as shown by increased antigen (HRP) binding, as illustrated in Fig. 13, than untreated wells in a concentration dependent manner. This demonstrates that these conjugates are a suitable and convenient means for the introduction of reactive carboxyl groups to solid phases.

Example 13

Preparation and Application of Fetuin-Hydrazide and Dinitrophenyl-Fetuin-Hydrazide Conjugates

Hydrophobic groups were introduced to fetuin (a glycoprotein) by reaction with 2,4-dinitrofluorobenzene. Fetuin was dissolved in 50 mM $NaHCO_3$ (pH 8.5) at 10 mg per ml and a 1/10 volume of 0.54 M 2,4-dintrofluorobenzene in DMSO was added. After a 2 hour incubation at 25°C the reaction halted by gel filtration in 50 mM acetate (pH 5.0), which removed unreacted 2,4-dinitrofluorobenzene and exchanged the buffer. DNP-fetuin or untreated fetuin at the same concentration was then oxidized by adding a 1/10 volume of 100 mM $NaIO_4$ in acetate buffer and incubating for 1 hour at 25°C in the dark. The reaction was halted by adding excess ethylene glycol

to consume unreacted $NaIO_4$ and the oxidized proteins were isolated by gel filtration into acetate buffer. Dry adipic acid dihydrazide was then added to the oxidized glycoproteins to give a final concentration of 100 mM and the mixtures were incubated for 16 hours at 25 °C; excess adipic acid dihydrazide was then removed by gel filtration into acetate buffer. Measurement of the absorbance at 365 nm indicates that there are 6 dinitrophenyl groups per fetuin molecule in the DNP-fetuin preparation. The overall synthesis routes are depicted in Fig. 14.

Serial dilutions of the conjugates were prepared in 50 mM acetate buffer (pH 5.0) and applied to polystyrene microtiter plates at 100 µl per well. After a 30 minute incubation at 25°C the plates were rinsed 3 times, 5 minutes each, with deionized water (200 µl per well) and 100 µl of either oxidized rabbit anti-peroxidase or untreated rabbit antiperoxidase (50 µg per ml in acetate buffer, diluted 1/10 with nonspecific rabbit IgG) were applied to the wells. After a 1 hour incubation at 25°C the plates were washed to remove unbound antiperoxidase and tested for peroxidase binding as described in Example 2. The results are reported in Fig. 15, wherein DNP-Fetuin-HZ: Ox = Dinitrophenyl-fetuin-hydrazide coated wells + oxidized IgG; Fetuin-HZ: Ox = Fetuin-hydrazide coated wells + oxidized IgG; DNP-Fetuin-HZ:N = Dinitrophenyl-fetuin-hydrazide coated wells + untreated IgG; and Fetuin-HZ: N = Fetuin-hydrazide coated wells + untreated IgG. Both fetuin-hydrazide and DNP-fetuin-hydrazide enhanced the antigen binding capacity of wells treated with oxidized IgG; wells treated with unoxidized IgG had essentially the same antigen binding capacity as uncoated wells. This demonstrates that polypeptides and/or glycoconjugates are suitable

polym rs for the introduction of reactive groups to solid phases. Wells treated with the DNP-fetuin-hydrazide conjugate showed an approximately 50% greater antigen binding capacity than those treated with fetuin-hydrazide conjugate, indicating that the addition of hydrophobic groups to this polymer enhances its binding to polystyrene.

Example 14

Preparation and Application of Monomeric Hydrophobe-Hydrazide Conjugates

Monomeric hydrophobe-hydrazide conjugates were prepared by dissolving or suspending 10 mg of n-octyl ß-D-glucopyranoside, octyl ß-D-thioglucopyranoside, n-dodecyl ß-D-glucopyranoside, or n-dodecyl ß-D-maltoside in 1 ml of 50 mM acetate, pH 5.0. Equimolar $NaIO_4$ was then added to each solution and the mixtures incubated for 1 hour at 25°C in the dark, at which point a 2 fold molar excess of adipic acid dihydrazide was added. After an additional 4 hours at 25°C the pH of the reaction mixtures was adjusted to 10 with 10 N NaOH and they were incubated at 5°C for 16 hours to precipitate sodium para-periodate. The overall synthetic route is depicted in Fig. 16.

Following centrifugation to remove precipitated sodium para-periodate, 100 µl of the supernatants were serially diluted with 50 mM acetate (pH 5.0) in a polystyrene microtiter plate. After an 1 hour incubation with the conjugate at 25°C the plates were rinsed for 5 minutes with deionized water and then 100 µl of oxidized rabbit antipe. oxidase (diluted 1:10 with oxidized nonspecific rabbit IgG, 50 µ per ml in acetate buffer) was added to the wells. After a 16 hour incubation at 5°C, the plates were washed with 100 mM Tris containing 0.05% Tween-20 (pH

7.5) to remove unbound anti-peroxidase and tested for peroxidase binding activity as described in Example 2. The results are illustrated in Fig. 17, wherein Dodecyl-G = oxidized N-dodecyl ß-D-glucopyranoside treated with adipic acid dihydrazide; dodecyl-M = oxidized N-dodecyl ß-D-maltoside treated with adipic acid dihydrazide; octyl-G = oxidized N-octyl ß-D-glucopyranoside treated with adipic acid dihydrazide; and octyl-thi-G = oxidized octyl ß-D-thioglucopyranoside treated with adipic acid dihydrazide. Wells coated with the monomeric hydrophobe-hydrazide conjugates showed significantly greater (approximately 6-7 fold increase) antigen binding than untreated wells, as shown by peroxidase activity. This demonstrates that monomeric forms of the hydrophobe-reactive group conjugates are a convenient means for the introduction of reactive groups to solid phases.

From the foregoing description, one skilled in the art can readily ascertain the essential characteristics of the invention and, without departing from the spirit and scope thereof, can adapt the invention to various usages and conditions. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient, and although specific terms have been employed herein, they are intended in a descriptive sense and not for purposes of limitation.

WHAT IS CLAIMED IS:

1. A composition of matter of general formula $(R^H-)_x$ A $(-X)_y$, wherein

R^H represents a hydrophobic moiety which develops substantial nonspecific interactions with a hydrophobic solid phase material;

A is an organic spacer group;

X represents a reactive group; and

each of x and y is an integer from 1 to about 1300.

2. A composition of matter according to claim 1, wherein said reactive group is selected from the group consisting of hydrazide, N-methylpyridyl, tresyl, aldehyde, haloacetyl, active esters of carboxylic acids, maleimido, imidate, epoxide, thiol, and dicarbonyl.

3. A composition of matter according to claim 2, wherein said reactive group is a hydrazide.

4. A composition of matter according to claim 2, wherein said reactive group is N-methylpyridyl.

5. A composition of matter according to claim 1, wherein said organic spacer group is a polymer selected from the group consisting of dextrans, hydrolyzed polyacrylamides, polyacrylic acids, polyvinyl alcohols and polypeptides.

6. A composition of matter according to claim 5, wherein said polymer is dextran.

7. A composition f matter according to claim 5, wherein said polymer is polyvinylalcohol.

8. A composition of matter according to claim 5, wherein said polymer is a hydrolyzed polyacrylamide.

9. A composition of matter according to claim 5, wherein said polymer is acrylic acid.

10. A composition for use in assays of a target molecule, comprising:

a composition of matter in accordance with claim 1;

a solid phase material associated with said hydrophobic groups; and

a ligand attached to said composition by said reactive groups.

11. A composition in accordance with claim 10, wherein said ligand is a protein.

12. A composition in accordance with claim 11, wherein said protein is a glycoprotein.

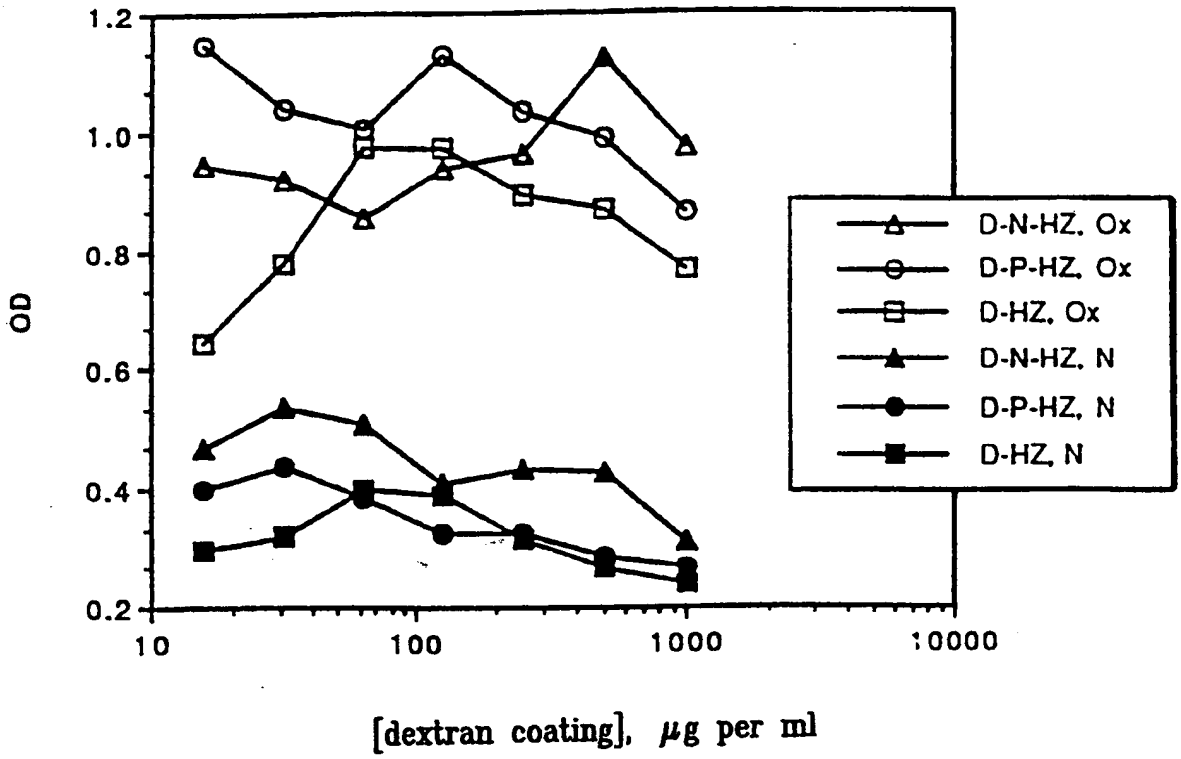
13. A composition in accordance with claim 12, wherein said glycoprotein is an immunoglobulin.

14. A composition in accordance with claim 10, wherein said solid phase material is a polystyrene microtiter plate.

15. A composition in accordance with claim 10, wherein said solid phase material is a polystyrene bead.

16. A composition in accordance with claim 10, wherein said solid phase material is a polyethylene bead.

17. A composition in accordance with claim 10, wherein said composition of matter is a modified polymer selected from the group consisting of dextrans, hydrolyzed polyacrylamides, polyacrylic acids, polyvinyl alcohols and polypeptides.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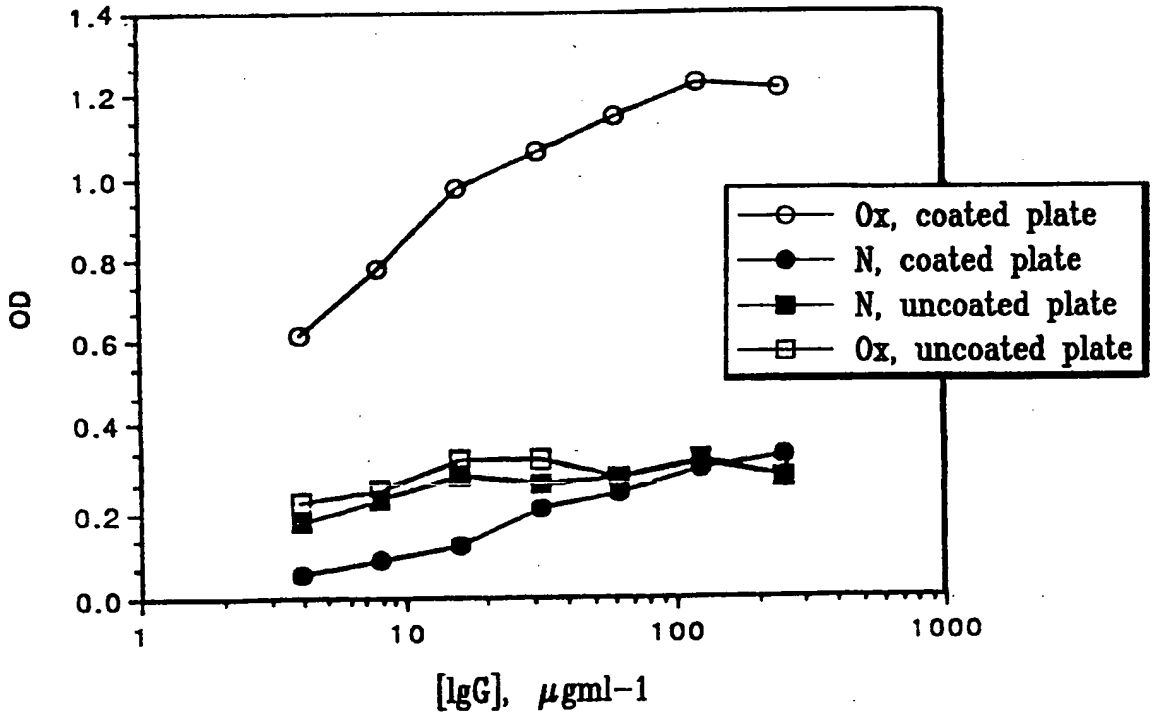
0.8
0.6
0.4
0.2
OD
10
100
1000
10000
D-N-HZ, Ox
D-P-HZ, Ox
D-HZ, Ox
D-N-HZ, N
D-P-HZ, N
D-HZ, N
[dextran coating], μg per ml

FIG. 1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1000
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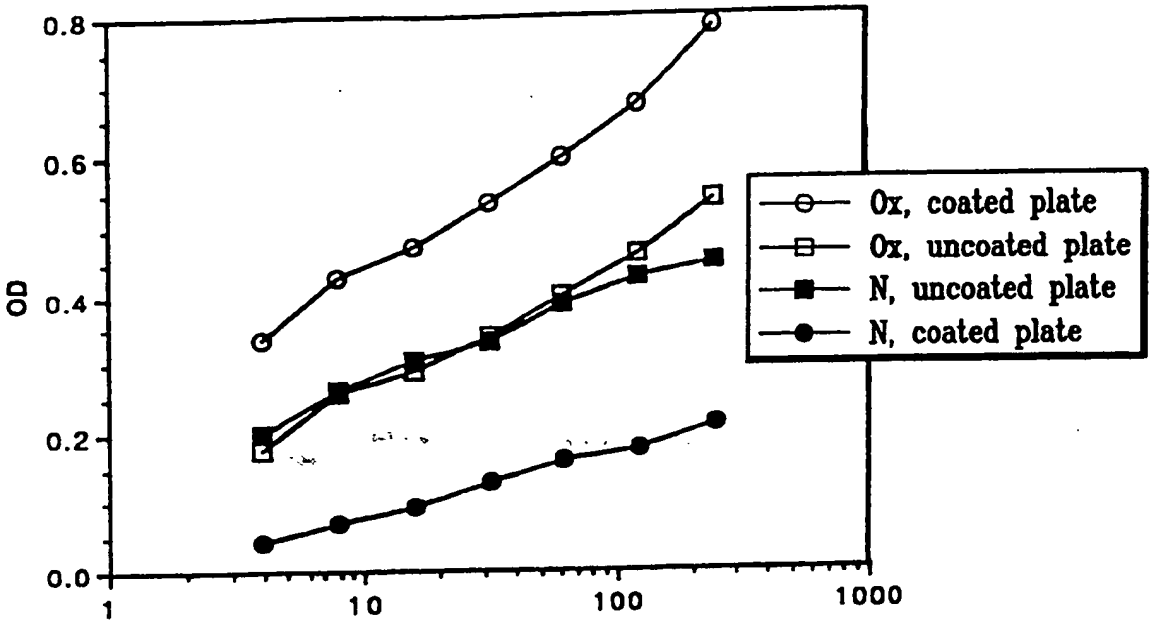
N, uncoated plate
Ox, uncoated plate
[IgG], μgml-1

FIG. 2A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100
1000
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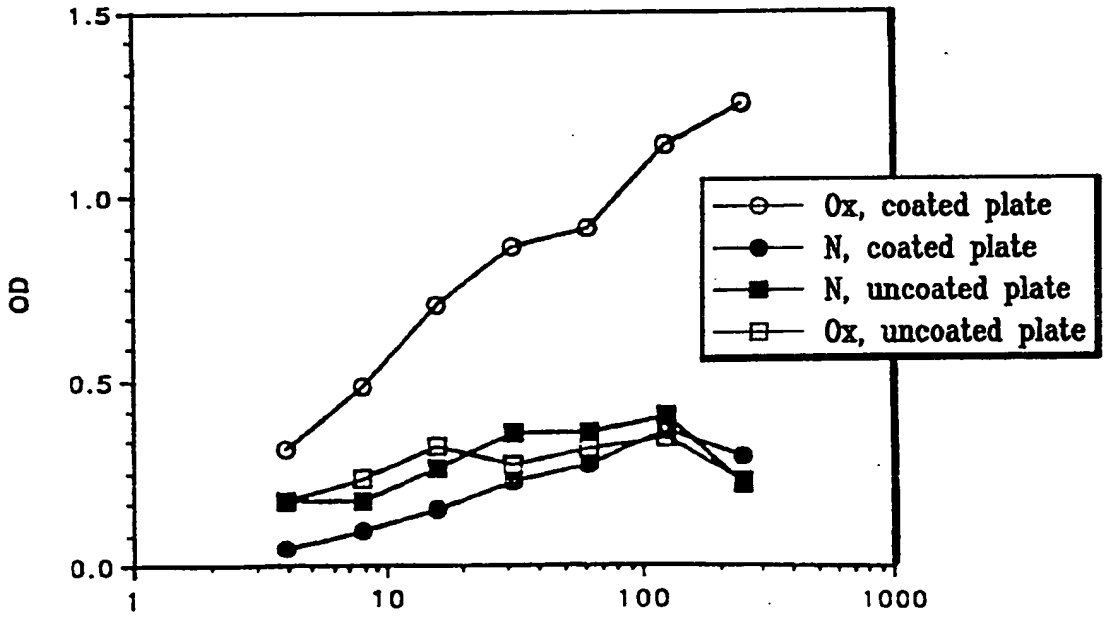
N, uncoated plate
N, coated plate
[IgG], μgml-1

FIG. 2B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0.0
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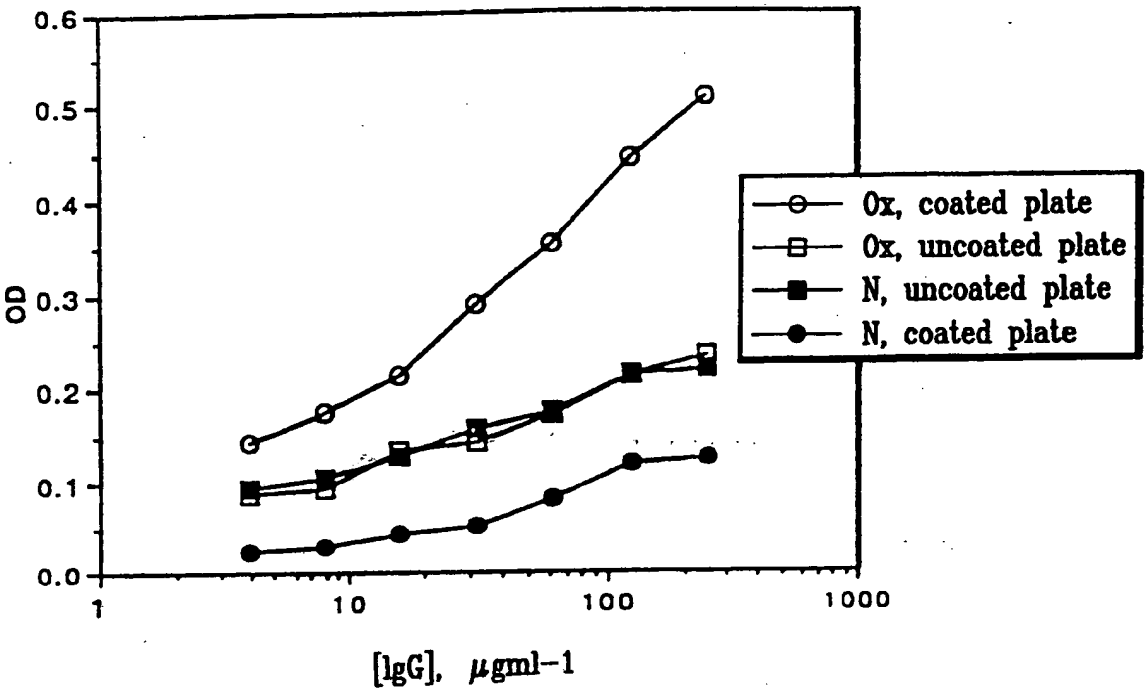
10
100
1000
Ox, coated plate
N, coated plate
N, uncoated plate
Ox, uncoated plate
[IgG], μgml-1

FIG. 2C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0.3
0.2
0.1
0.0
OD
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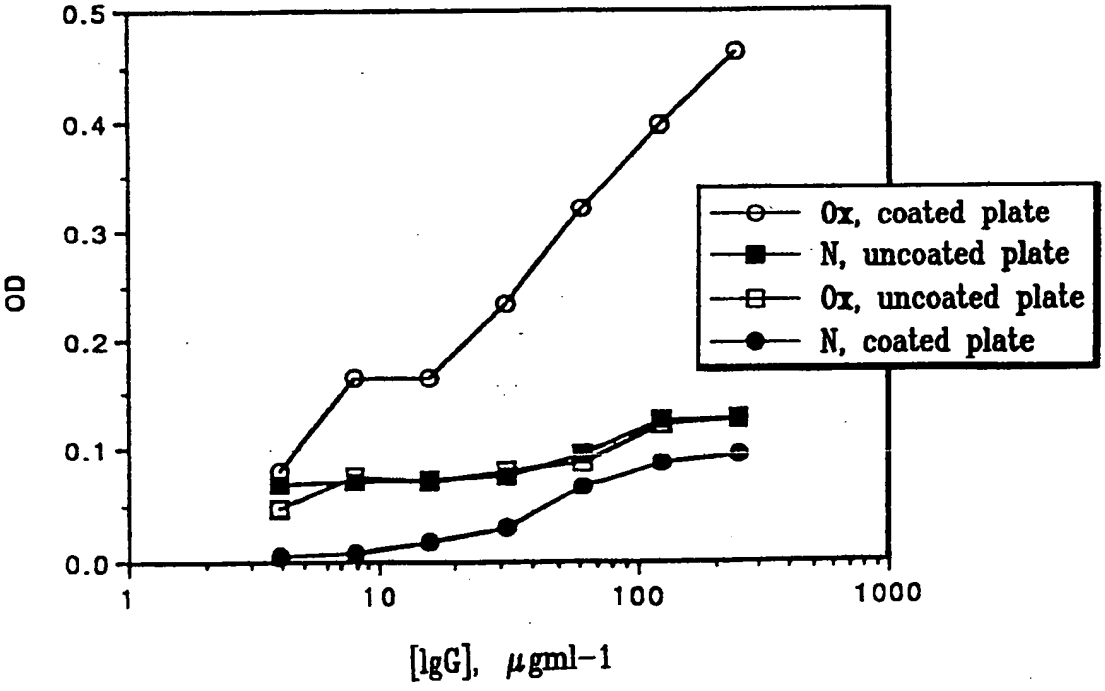
1000
[lgG], μgml-1
Ox, coated plate
Ox, uncoated plate
N, uncoated plate
N, coated plate

FIG. 2D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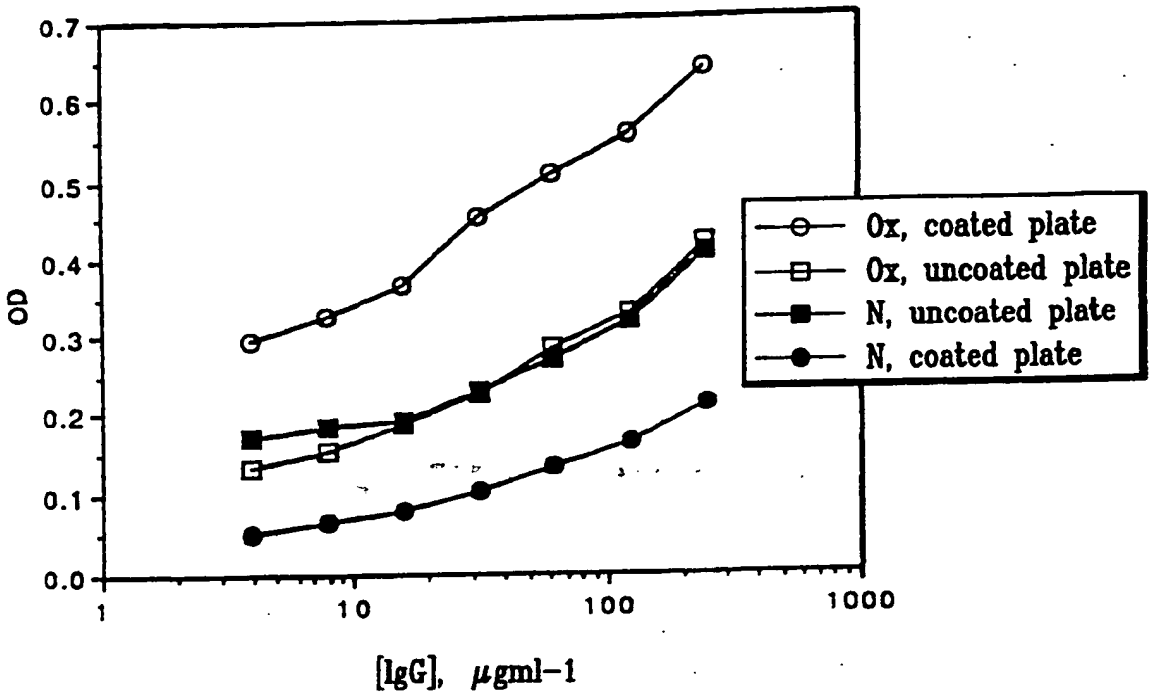
0.4
0.3
0.2
0.1
0.0
OD
1
10
100
1000
[lgG], μgml-1
Ox, coated plate
N, uncoated plate
Ox, uncoated plate
N, coated plate

FIG. 2E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0.5
0.4
0.3
0.2
0.1
0.0
OD
1
10
100
1000
[IgG], μgml-1
Ox, coated plate
Ox, uncoated plate
N, uncoated plate
N, coated plate

FIG. 2F

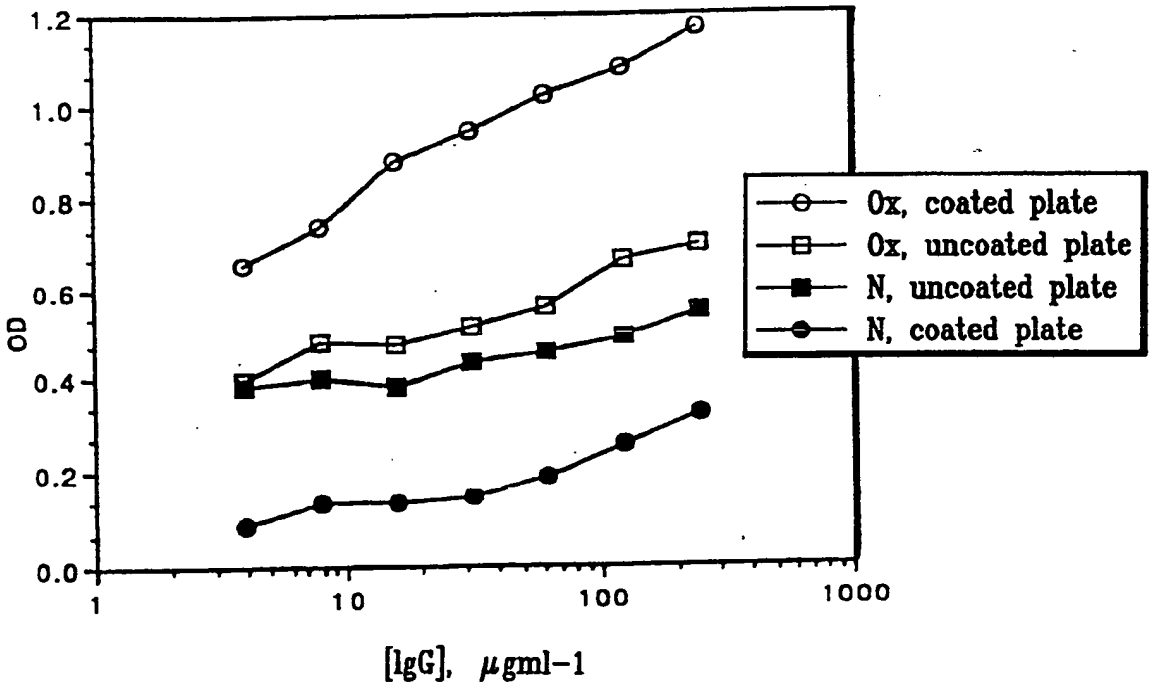
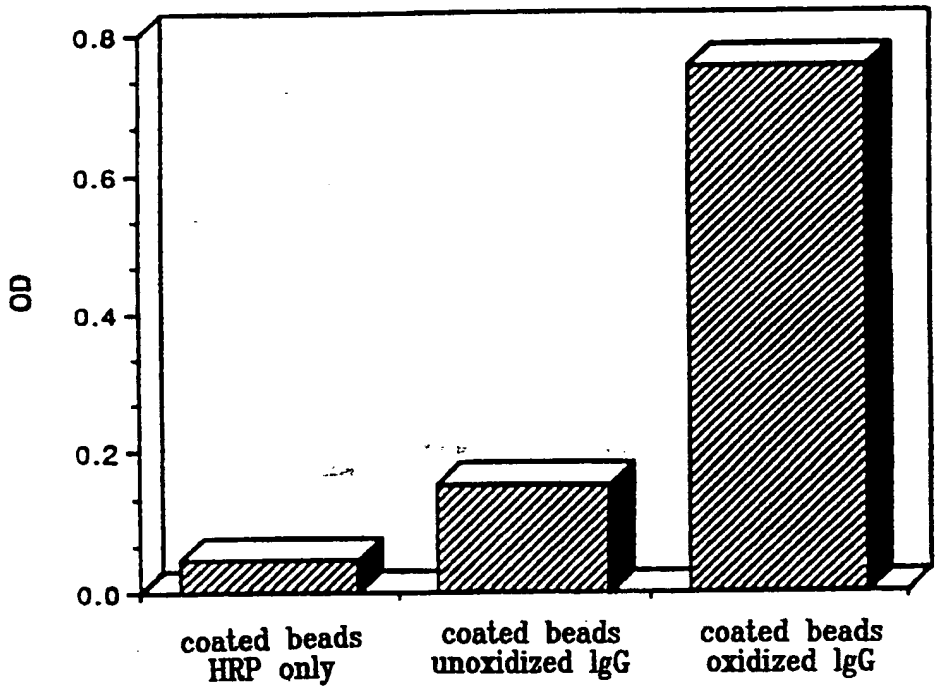

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD
1
10
100
1000
[IgG], μgml-1
Ox, coated plate
Ox, uncoated plate
N, uncoated plate
N, coated plate

FIG. 2G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0.4
0.2
0.0
OD
coated beads HRP only
coated beads unoxidized IgG
coated beads oxidized IgG

FIG. 3

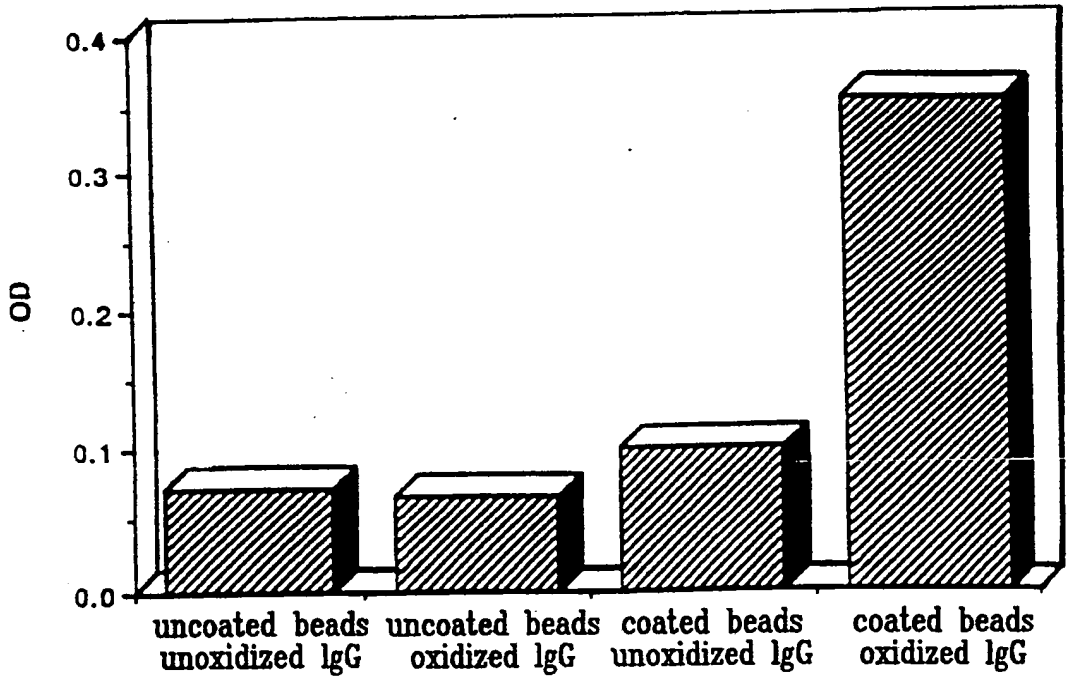
0.4
0.3
0.2
0.1
0.0
OD
uncoated beads unoxidized IgG
uncoated beads oxidized IgG
coated beads unoxidized IgG
coated beads oxidized IgG

FIG. 4A

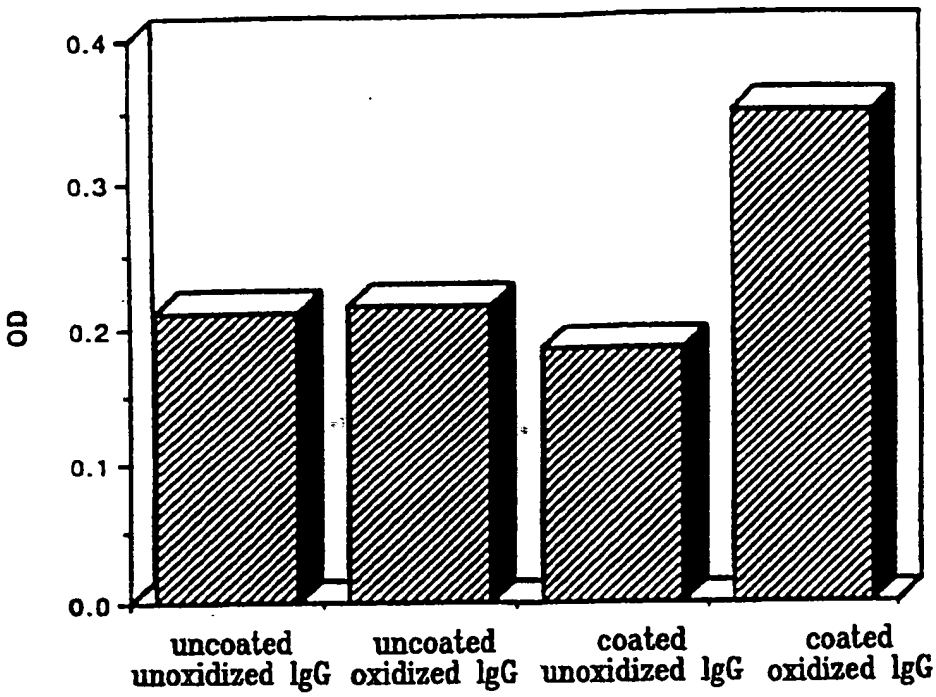
0.4
0.3
0.2
0.1
0.0
OD
uncoated unoxidized IgG
uncoated oxidized IgG
coated unoxidized IgG
coated oxidized IgG

FIG. 4B

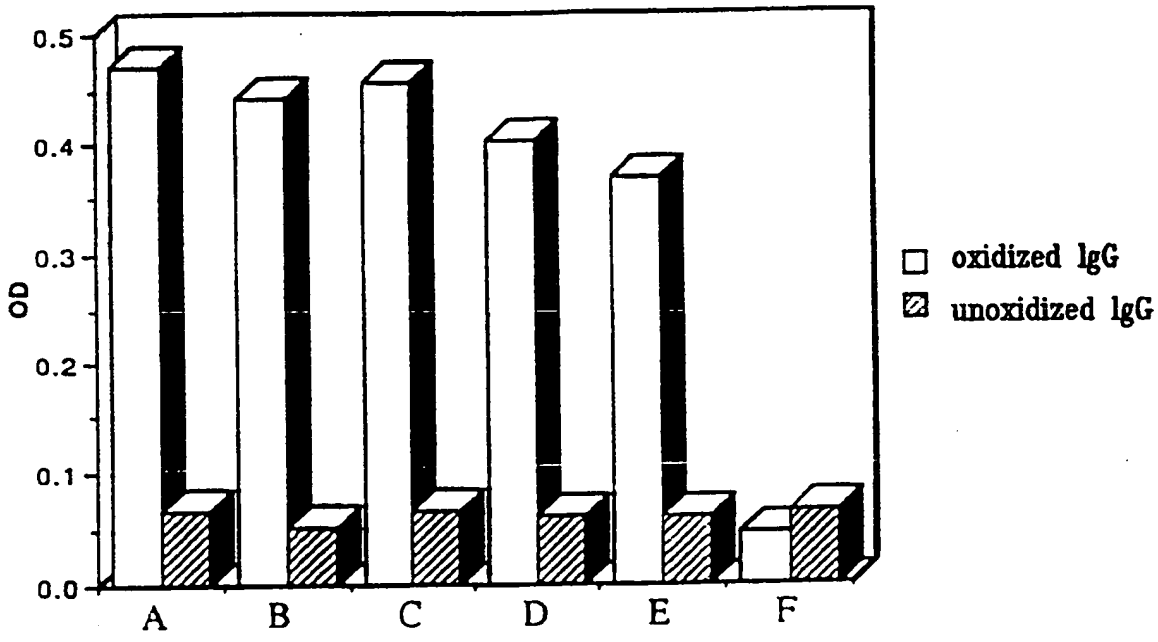
0.5
0.4
0.3
0.2
0.1
0.0
OD
A
B
C
D
E
F
oxidized IgG
unoxidized IgG

FIG. 5

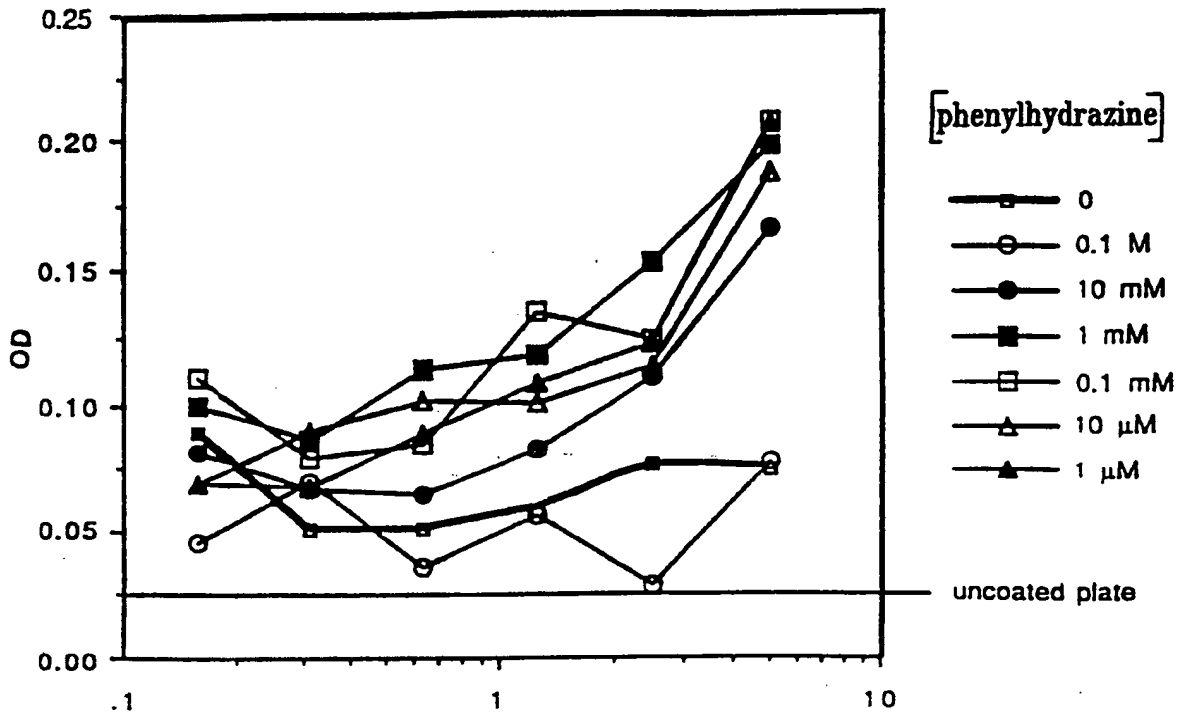
0.25
0.20
0.15
0.10
0.05
0.00
OD
.1
1
10
[coating] mg per ml
[phenylhydrazine]
0
0.1 M
10 mM
1 mM
0.1 mM
10 µM
1 µM
uncoated plate

FIG. 6

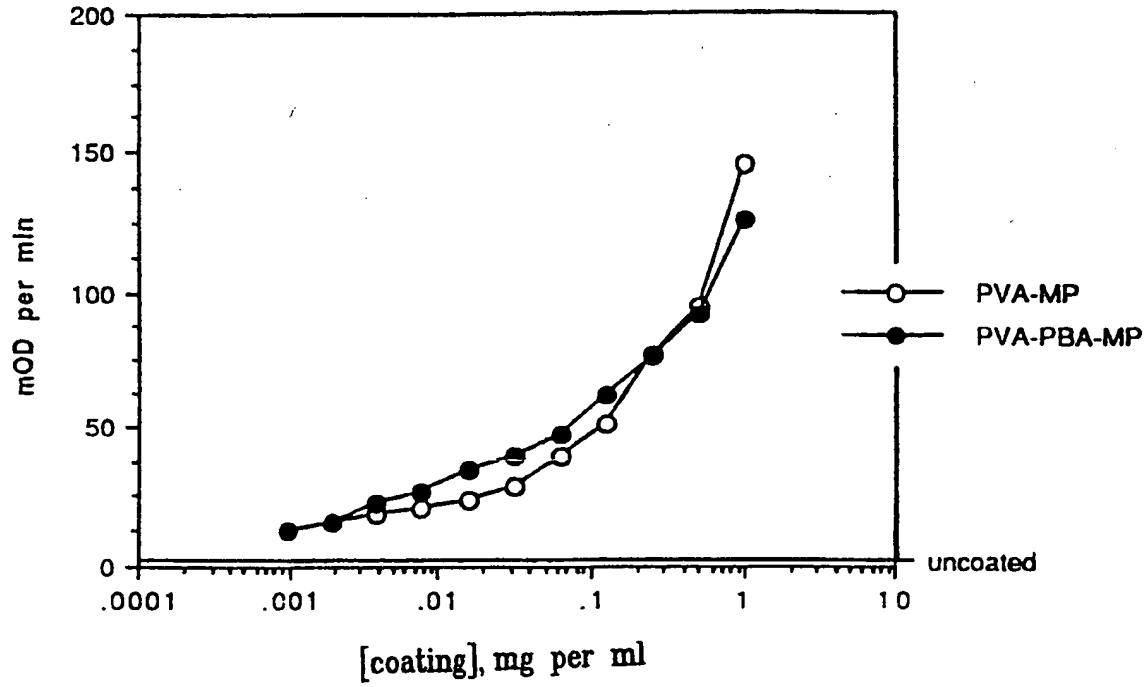
200
150
100
50
0
mOD per min
.0001
.001
.01
.1
1
10
[coating], mg per ml
PVA-MP
PVA-PBA-MP
uncoated

FIG. 7

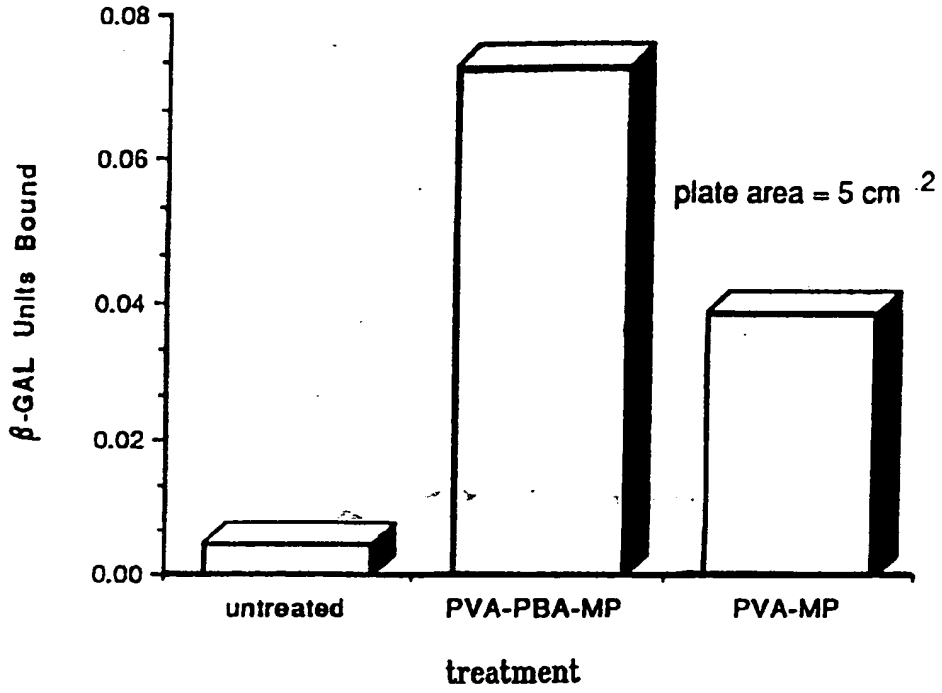
0.08
0.06
0.04
0.02
0.00
β-GAL Units Bound
plate area = 5 cm 2
untreated
PVA-PBA-MP
PVA-MP
treatment

FIG. 8

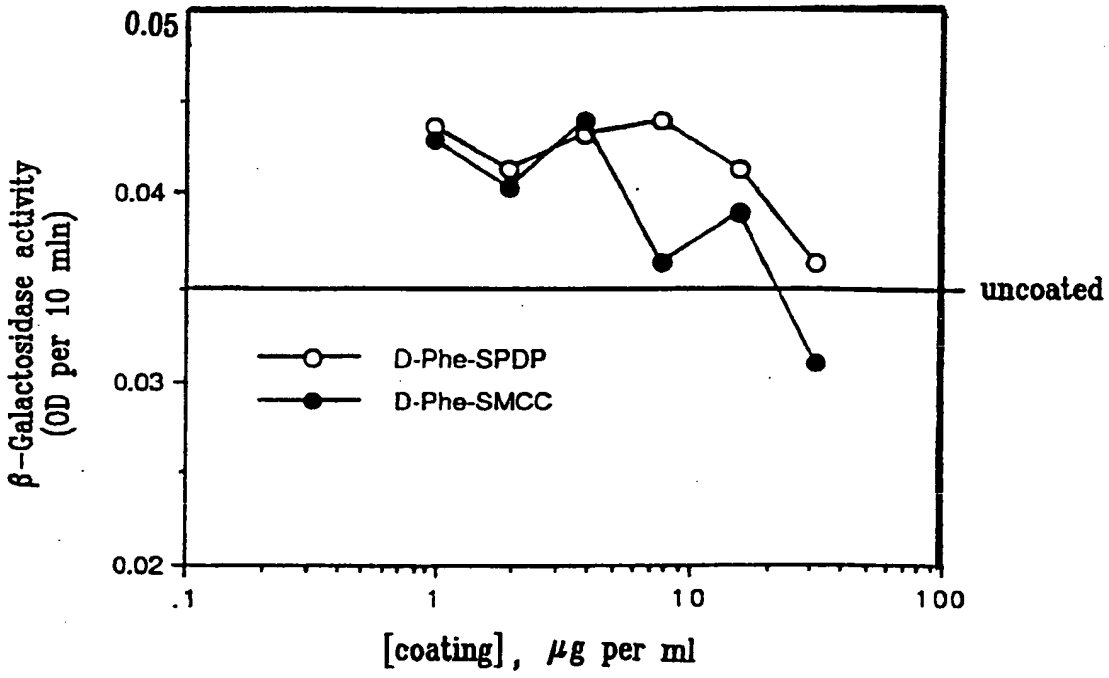
0.05
0.04
0.03
0.02
β-Galactosidase activity (OD per 10 min)
uncoated
D-Phe-SPDP
D-Phe-SMCC
.1
1
10
100
[coating], μg per ml

FIG. 10

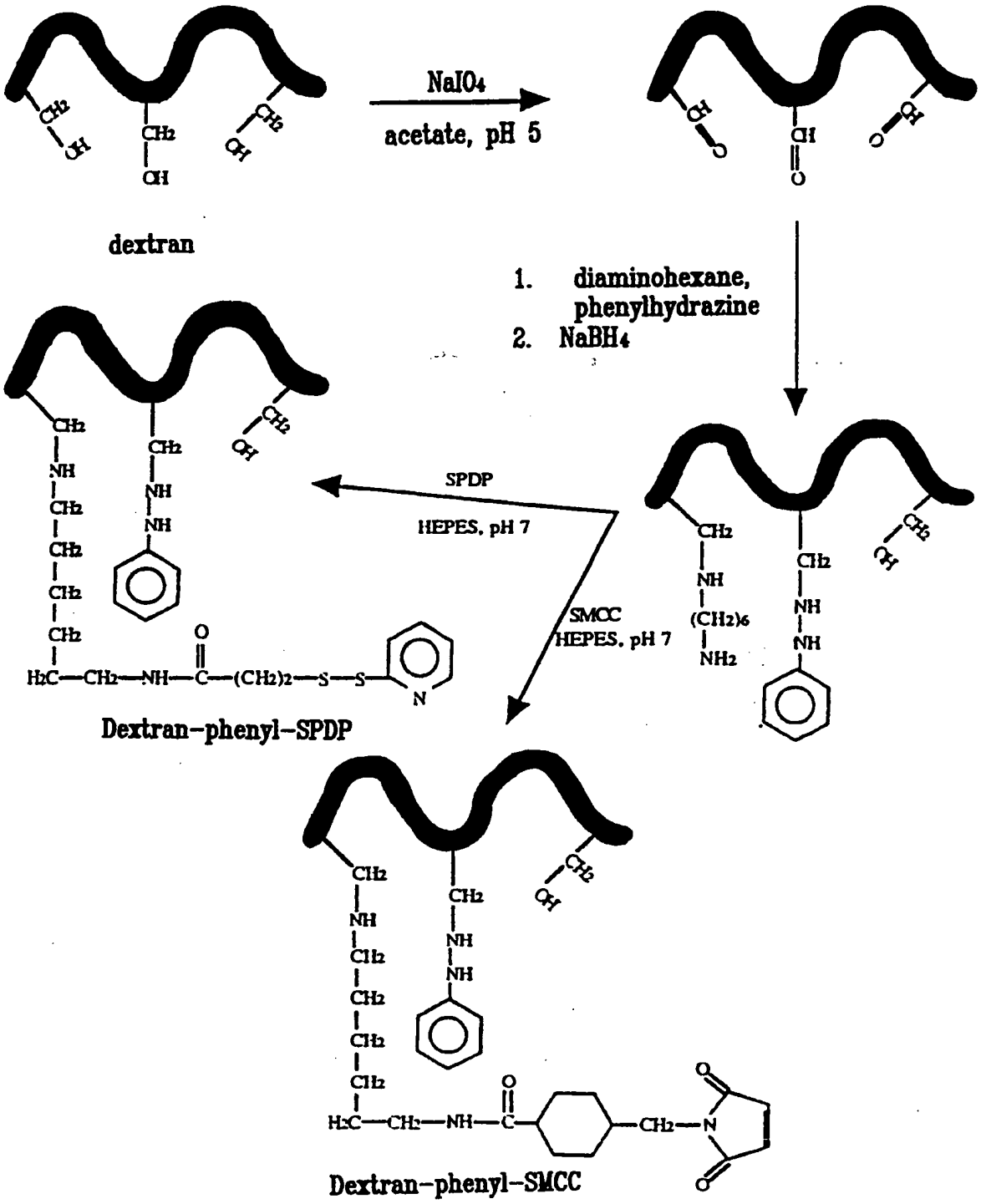
NaIO4
acetate, pH 5
dextran
1. diaminohexane, phenylhydrazine
2. NaBH4
SPDP
HEPES, pH 7
SMCC
HEPES, pH 7
Dextran-phenyl-SPDP
Dextran-phenyl-SMCC

FIG. 9

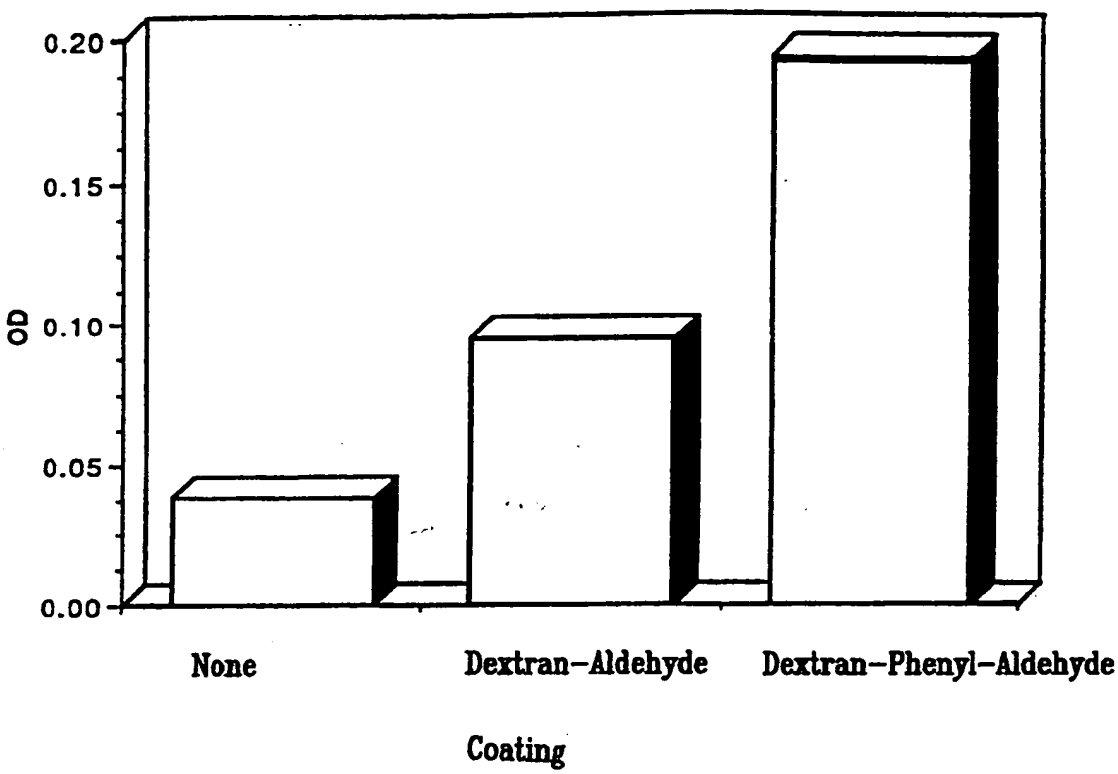
0.20
0.15
0.10
0.05
0.00
OD
None
Dextran-Aldehyde
Dextran-Phenyl-Aldehyde
Coating

FIG. 11

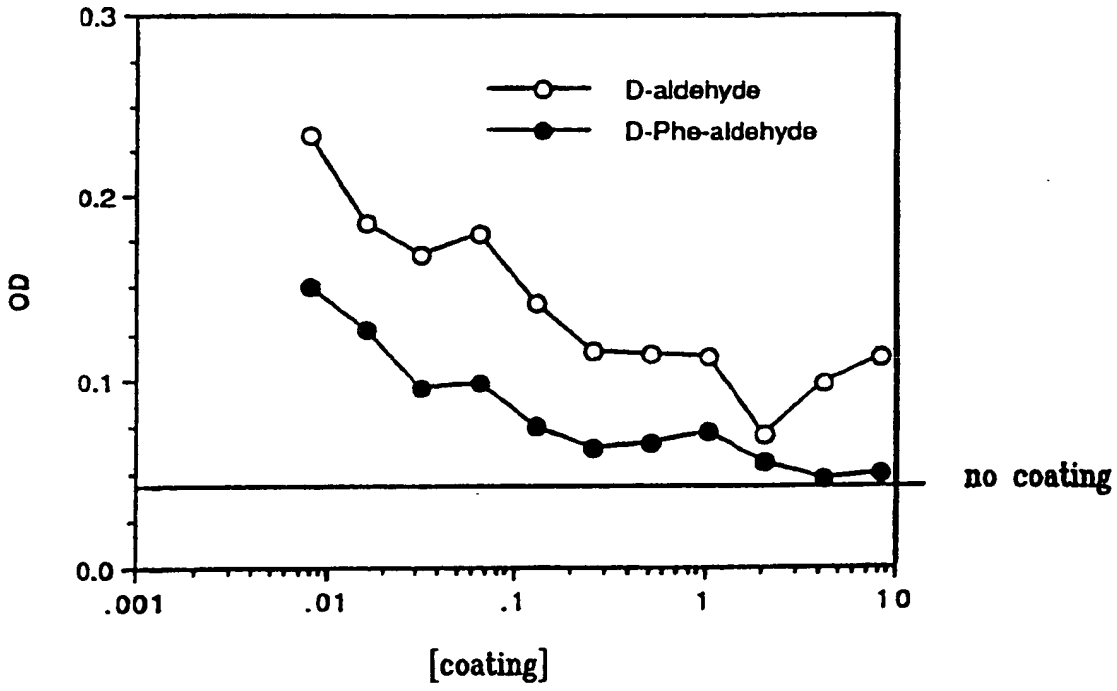
0.3
0.2
0.1
0.0
OD
D-aldehyde
D-Phe-aldehyde
no coating
.001
.01
.1
1
10
[coating]

FIG. 12

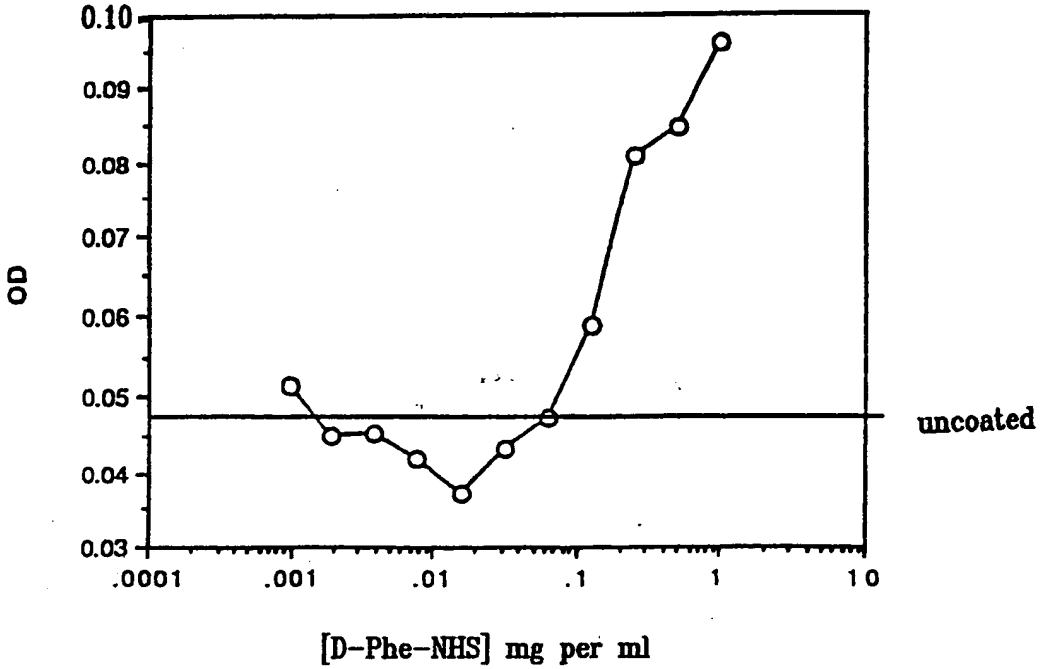
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
OD
.0001
.001
.01
.1
1
10
uncoated
[D-Phe-NHS] mg per ml

FIG. 13

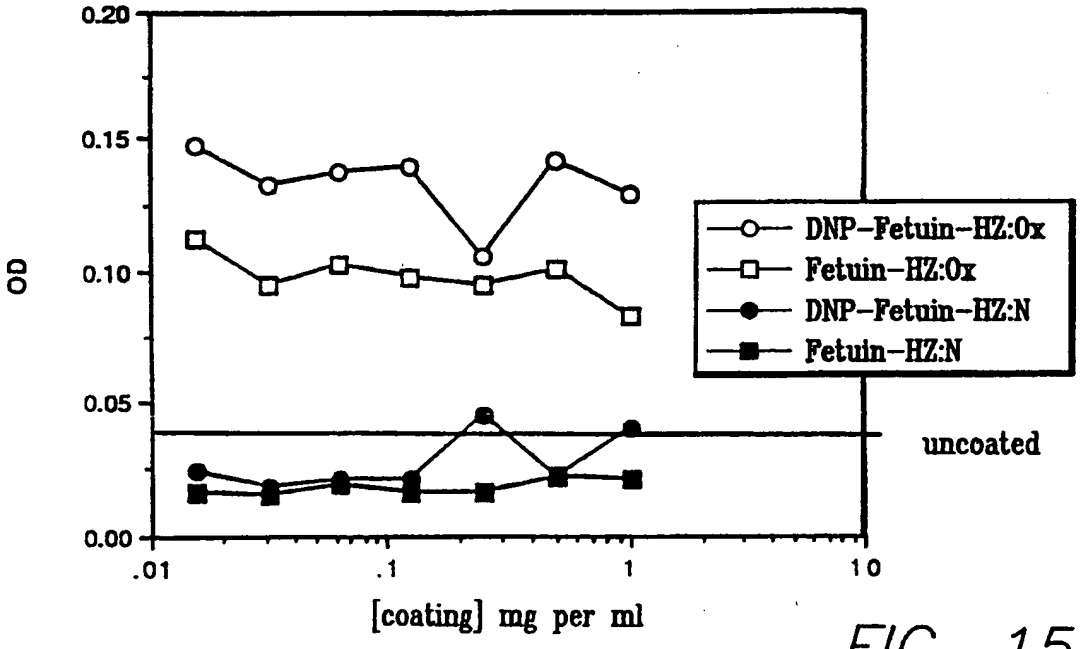
0.20
0.15
0.10
0.05
0.00
OD
.01
.1
1
10
DNP-Fetuin-HZ:Ox
Fetuin-HZ:Ox
DNP-Fetuin-HZ:N
Fetuin-HZ:N
uncoated
[coating] mg per ml

FIG. 15

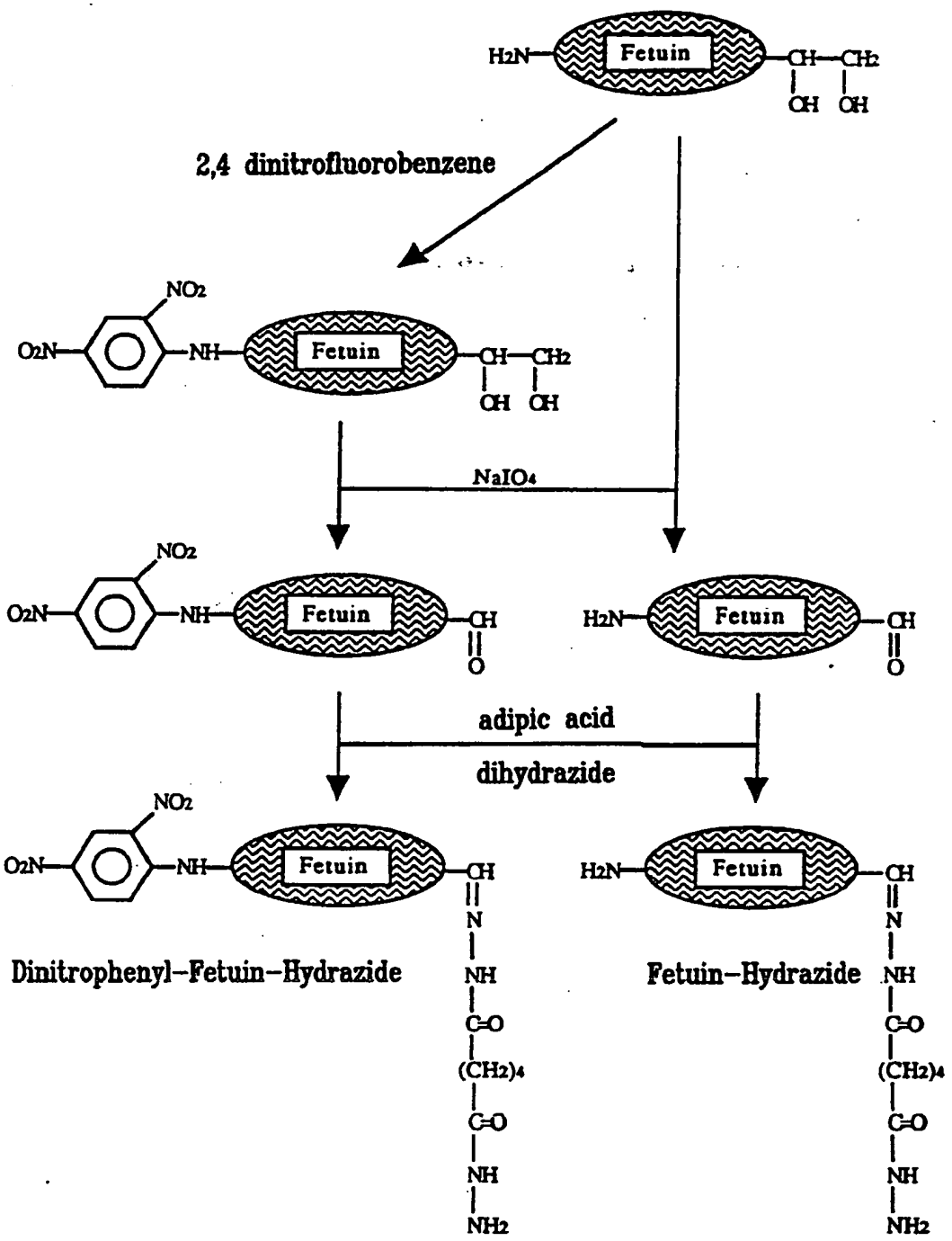
H2N
Fetuin
CH
CH2
OH
OH
2,4 dinitrofluorobenzene
NO2
O2N
NH
NaIO4
adipic acid
dihydrazide
Dinitrophenyl–Fetuin–Hydrazide
Fetuin–Hydrazide
N
C=O
(CH2)4
NH2
O

FIG. 14

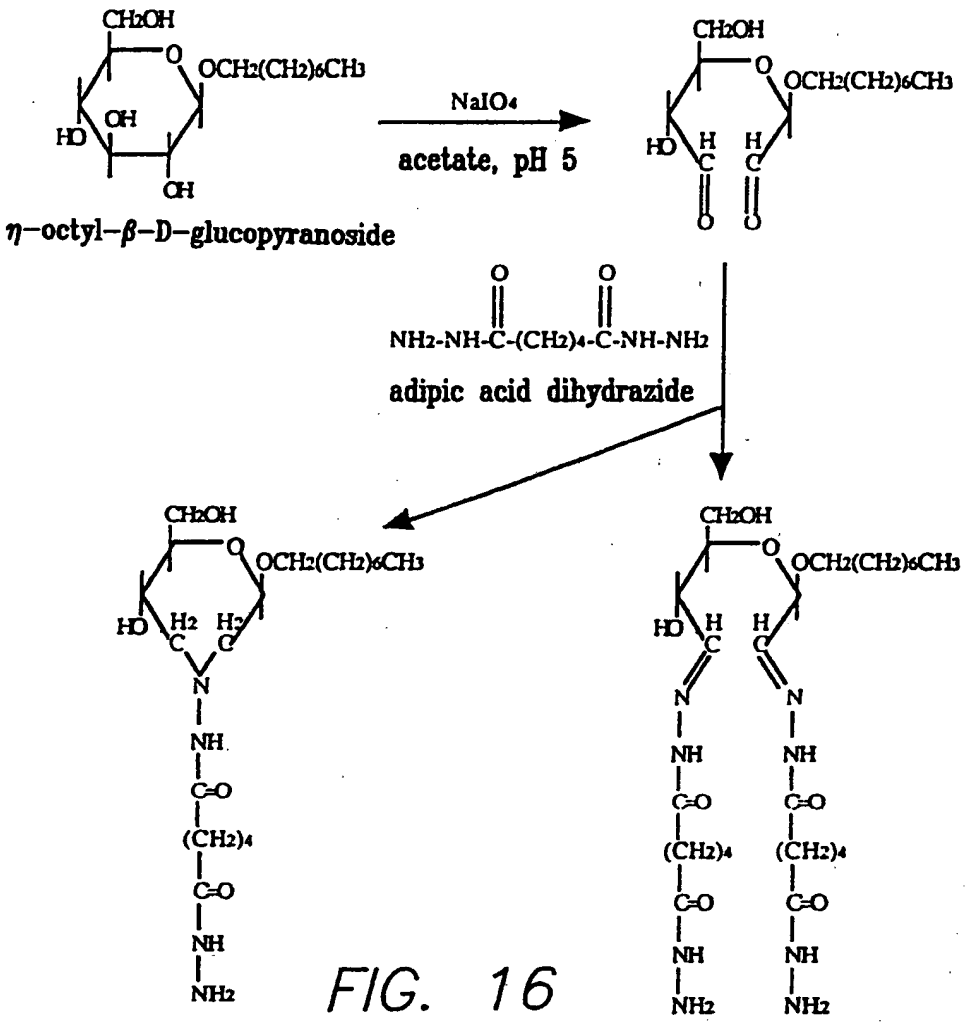
CH2OH
OCH2(CH2)6CH3
OH
HO
OH
η–octyl–β–D–glucopyranoside
NaIO4
acetate, pH 5
NH2-NH-C-(CH2)4-C-NH-NH2
adipic acid dihydrazide
NH
C=O
(CH2)4
NH2

FIG. 16

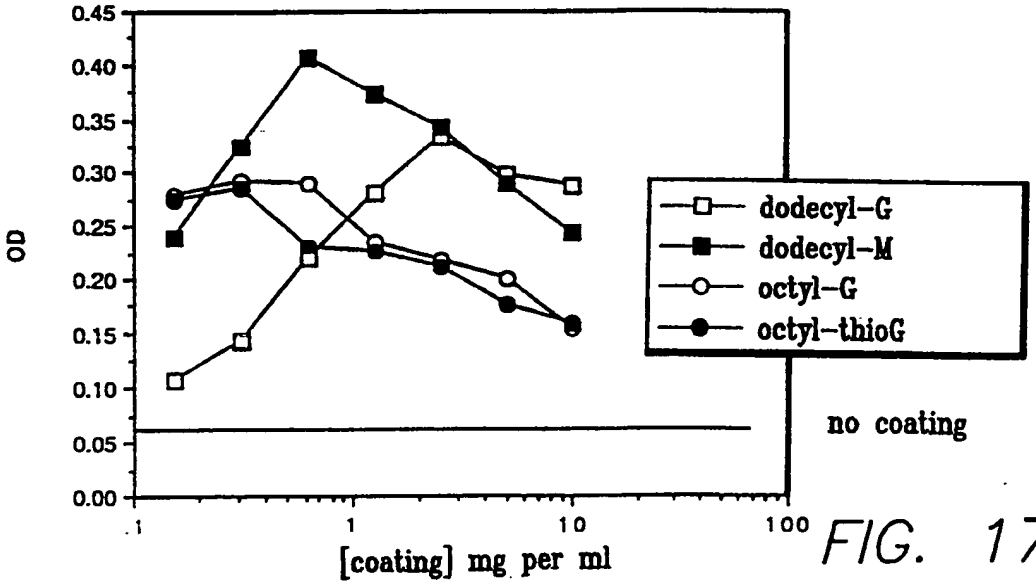
OD
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
.1
1
10
100
[coating] mg per ml
dodecyl–G
dodecyl–M
octyl–G
octyl–thioG
no coating

FIG. 17